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[illegible] ALKALI RESERVE OF NORMAL, PREGNANT, AND FETAL SHEEP *Donald H. Barron*

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The new format of the *Yale Journal* has been designed by Mr. Charles E. Hammond, a candidate for the degree of Master of Fine Arts, Yale University. Mr. Hammond is at present a designer on the staff of the Virginia Museum of Fine Arts, Richmond, Virginia.

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THE LIMBIC SYSTEM: A STUDY OF THE VISCERAL BRAIN IN PRIMATES AND MAN*

INTRODUCTION

The subject of the limbic system is old anatomically, but very new from the point of view of our knowledge of its function—indeed, so new that many of the more important disclosures have been made within the last two years. These recent advances have a direct bearing on many branches of clinical medicine and are of immediate importance both to the general physician and to the neurologist. The limbic system, or "visceral brain," as it has recently been designated,[80] was first defined by Paul Broca in 1878,[6] and I give his original definition:

> Le nom de *circonvolution limbique* que j'ai adopté indique les rapports constants de cette circonvolution avec le limbe de l'hemisphère; il n'implique aucune théorie; n'exprimant pas une forme determinée; il est applicable à tous les cerveaux des mammifères, à ceux qui ont un vrai corps calleux comme à ceux dont le corps calleux est nul ou rudimentaire (*lyencéphales* d'Owen), à ceux qui ont un vrai lobe olfactif, comme à ceux dont le lobe olfactif n'est qu'à l'état de vestige. Enfin, il a l'advantage de permettre de designer sans changement d'adjectif les parties qui se rattachent à la description de cette circonvolution: le grand *lobe limbique, la scissure limbique, l'arc limbique supérieur ou inférieur, etc.*†

Broca was thus primarily concerned with the gross morphological relations of the limbic lobe, and it remained for Ramón y Cajal to give the first adequate analysis of the finer structure and connections of this phylogenetically ancient part of the forebrain. Cajal[39] was aware that all parts of the limbic system were interconnected, the pyriform with the hippocampus and the cingulate with the parasplenial and subcallosal gyri, and that the limbic system as a whole projected to subcortical nuclei such as the amygdala, anterior thalamic nuclei, hypothalamus, and basal ganglia. He was not fully aware, however, of the major afferent connections of the limbic

---

* Based on a lecture read on September 10, 1953 at Lisbon during the Vth International Neurological Congress. I am grateful to Drs. Delgado, MacLean, and Rosvold for their critical scrutiny of the text.

† It is now recognized that certain adjacent structures such as the tip of the temporal lobe and posterior orbital gyri belong functionally to the limbic system. J.F.F.

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system from spinal cord and midbrain. Indeed, the afferent supply of the limbic system has been further clarified only within the last year.

The reason why the limbic system has now assumed such conspicuous importance lies in the fact that it influences many phases of autonomic function as well as patterns of emotional behavior. It perhaps also serves to integrate the higher intellectual functions of the neocortex with the more primitive visceral functions. Since 1950 these relations have been notably clarified by Henri Gastaut[19, 20, 21, 49] of Marseilles, Jean Colle[7] of Louvain, Paul Dell[11, 12] of Paris, Birger Kaada[26, 27] of Oslo, C. B. B. Downman[13] of London, V. E. Amassian[2, 3] of Seattle, and a group at Yale: Paul MacLean,[31, 32] José M. R. Delgado,[9, 10] Karl Pribram,[36, 37] and H. E. Rosvold.[9, 38] As a result of their studies, the limbic lobe has emerged as a distinct functional entity concerned primarily with the regulation of visceral organs and elaboration of affective behavior, while the neocortex is recognized as presiding in large measure over conscious sensory perception and the more purely intellectual functions of the brain.[17]

AFFERENT CONNECTIONS OF THE LIMBIC SYSTEM

The classic spinothalamic system arising in the spinal cord and projecting to the ventral nuclei of the thalamus, and thence passing in precise topographical fashion to the cerebral cortex, was long thought to be the chief, if not the only, afferent supply of the forebrain. However, Kappers *et al.*[29] and others described spino-hypothalamic connections in fish and certain other lower forms. They pointed out that this ascending system, completely independent of the spinothalamic system, passed to the hypothalamus via the mammillary peduncle. In 1935 Ibañez[25] of the Cajal Institute found ascending peduncle fibers from the ventral tegmental nucleus, and in 1936 Tello[41] demonstrated in the 14 mm. cat fetus the entire course of the mammillary peduncle from the ventral tegmental nucleus to the mammillary body in a single sagittal section. In 1940 Bronk[6] and his associates found electrical evidence of an ascending system from medulla to hypothalamus in higher animals. Now, thanks to the investigations of Dell,[11] Downman,[13] Amassian,[2, 3] and Aidar *et al.*,[1] we know that two large extralemniscal systems of autonomic afferents ascend from the spinal cord through medulla and midbrain to hypothalamic and adjacent nuclei, and that some of the ascending medullary connections project directly to the limbic system of the forebrain. One system of fibers is slow, and these appear to terminate subcortically in hypothalamus and adjacent structures; some of the more rapid fibers proceed directly to the posterior orbital gyrus.

A few months ago French, Verzeano, and Magoun[14] disclosed (through the use of evoked electrical potentials) that a large portion of the extralemniscal system terminates in the reticular formation and projects from there to various parts of the forebrain, including the limbic cortex (Fig. 1).

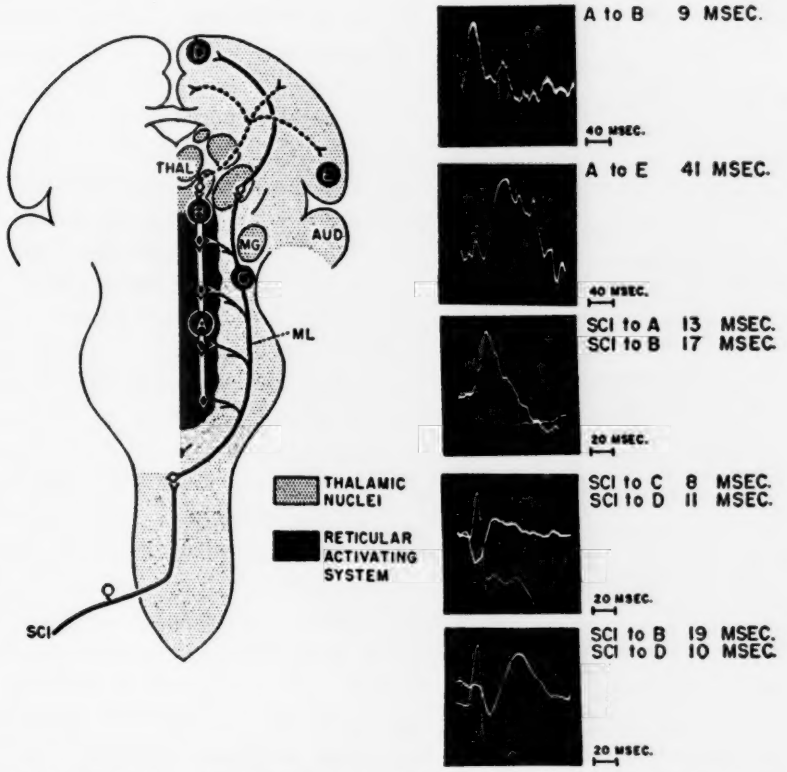

FIG. 1. Conduction time of evoked potentials to the brain stem and cortex. The left-hand figure illustrates recording points of evoked potentials on stimulation of the sciatic nerve. The photographs of the oscilloscope recordings on the right side show relative latencies at the appropriate points lettered. The upper two records show evoked potentials resulting from stimulation of the caudal reticular formation as recorded in the medial thalamus and the frontal association cortex. (From French, Verzeano, and Magoun, see Ref. 14.)

I give emphasis to this newly recognized system of afferent fibers because the fibers are severed in the now classical lobotomy procedure of Moniz and Lima, and I believe that this circumstance accounts for the dramatic relief which a lobotomy may confer on a patient suffering from intractable pain. When these fibers are severed, pain communications to the sensorium from various visceral organs are interrupted, with the result that fewer

pain impulses can impinge upon the sensorium in a given time. The patient may still be conscious of pain because all pain fibers cannot be severed, but pain ceases to disturb the patient because the number of intact pain fibers has been curtailed. French *et al.* also point out that this extralemniscal system is selectively blocked by anesthetic agents, as are cortical internuncials, long before there is interruption of the spinothalamic system, and they suggest that this may be responsible for the anesthetic state.

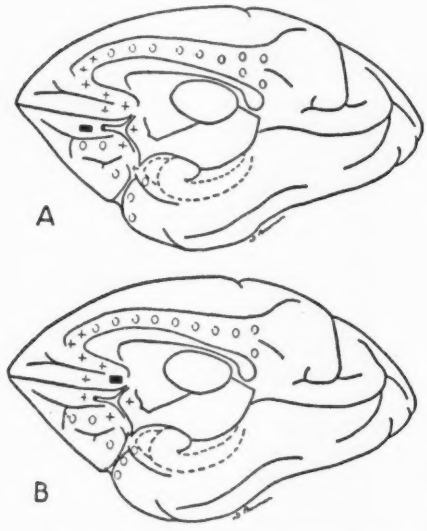

FIG. 2. Effects of local application of strychnine on orbital and subcallosal areas (site of application indicated by black rectangle). A, medial orbital area; B, subcallosal area: + = firing; 0 = no firing. These two areas have been identified as FL cytoarchitecturally by von Bonin and Bailey. (From Pribram, Lennox, and Dunsmore, see Ref. 36.)

In this connection it is of great interest that MacLean *et al.*[34] have obtained conspicuous electrical responses of the pyriform area to noxious stimuli (pinching ear or foot), indicating that pain stimuli are, in fact, transmitted to the limbic system. The reactions were in every way similar to those evoked by olfactory stimuli.

INTERCONNECTIONS WITHIN THE LIMBIC SYSTEM

Because of the fact that many interconnecting fibers within the different parts of the limbic system are unmyelinated, it has been difficult to trace intralimbic projections anatomically; but the physiological method of strychnine neuronography has, in the hands of MacLean,[35] Pribram,[36] and others, been highly successful in establishing functional interrelations. As indicated in the diagram (Fig. 2), when a small rectangle of paper soaked in strychnine is placed on the anterior cingulate, there is extensive "firing" of the cingulate gyrus as well as a part of the extralimbic cortex extending onto the lateral convexity. By such a technique it is possible to show regions of interrelated cortex. In the frontotemporal region, for example, we find that the posterior orbital, anterior insular, temporal polar, and pyriform areas are all interconnected. Such regional relationships are shown graphically in the next composite illustration (Fig. 3). Already many of the connections postulated by the strychnine method have been demonstrated ana-

tomically by Glees *et al.*[22] and also Wall *et al.*[48] From these disclosures one can only conclude that the various parts of the limbic system are in close functional interrelation.

ELECTRICAL STIMULATION OF THE LIMBIC SYSTEM

In 1938 Bailey and Bremer[4] found that stimulation of the central end of the vagus nerve caused marked electrical changes in the posterior orbital gyrus, suggesting that the vagus had discrete cortical representation. Two years later Bailey and Sweet[4] reported that stimulation of the posterior orbital gyrus gave rise to changes in blood pressure and gastric motility and that it also inhibited respiratory movements, as W. G. Spencer[40] had disclosed in 1894. In 1949 Kaada[29] and his associates observed similar vascular and respiratory responses from direct stimulation of the temporal pole, the insula, orbital surface, and cingulate gyrus, and they ascertained, furthermore, that each of these various structures giving like responses reacts best to the same type of electrical stimulus, the optimal parameters being square-wave pulses of 10 msec. duration at 40/sec. at low intensity, i.e., 3-8 volts. The continuous excitable region yielding these responses is indicated in the accompanying figure (Fig. 4). Kaada[27] has since elaborated upon these studies in his thesis, extending them to cats, dogs, monkeys, and a young chimpanzee; and he has also extended the regions stimulated to include all parts of the limbic system and the closely related subcallosal, orbito-insular, pyriform and temporal cortex, together with the hippocampus, fornix, and amygdala. He concludes that all viscera having autonomic

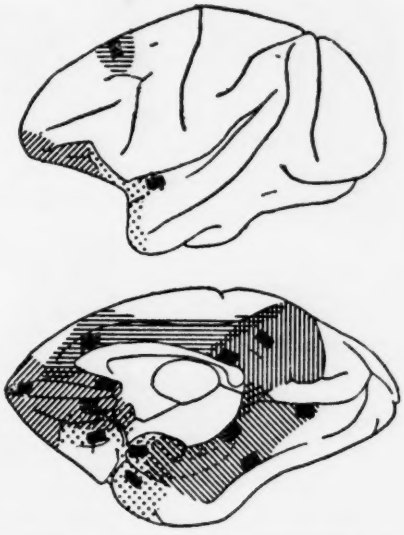

FIG. 3. Schematic representation of the lateral (top figure) and mediobasal (bottom figure) surface of the macaque's brain, showing regions of the phylogenetically old and new cortex, which on the basis of strychninization studies are inferred to have a close functional relationship. The "primitive" cortex (visceral brain) completely encircles the hilus of the hemisphere. Black rectangles within the variously shaded regions indicate the portions of the "primitive" and "new" cortex that were strychninized and found to be reciprocally related. The respective shadings correspond to the extent of cortex fired by the "primitive" cortex within each region. Note the overlap between regions. The extent of the firing into the outlying neopallium by the designated neocortical areas is not shown. (From MacLean, see Ref. 31.)

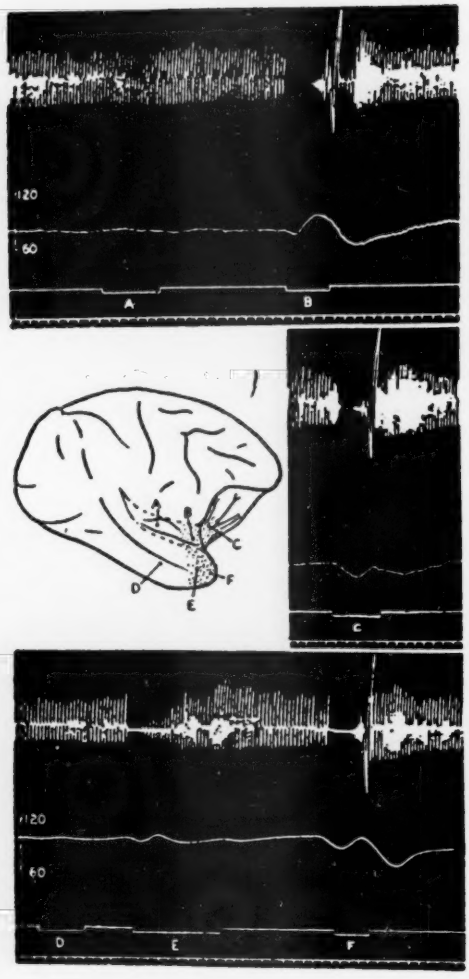

FIG. 4. Respiratory (upper record) and blood pressure responses (lower record) resulting from electrical stimulation of points corresponding to designation on brain map. Insula (A and B) visualized by separation of temporal and frontoparietal operculum. Responsive regions indicated by dots. Respiratory movements through tracheal cannula. Stroke upwards indicates expiration. Parameters of stimulation: Int. = 5.5 volts, F. = 39.5 per sec., sigma = 10 msec. Time 5 sec. (From Kaada, Pribram, and Epstein, see Ref. 28.)

innervation — i.e., heart and blood vessels, gut, pupil, lachrymal gland, pilomotor system, etc.—have representation in the limbic system and related medial structures, and that the limbic system is to be looked upon as the primary autonomic center of the forebrain.

Effects of limbic stimulation in conscious animals. Through the use of implanted electrodes, Gastaut, MacLean, Delgado, and their various associates, working at first (1950-51) quite independently, find that stimulation of the amygdala causes striking behavioral changes in dogs, cats, and monkeys. To quote MacLean and Delgado[83]:

> From widespread and interspersed points of cat and monkey in the frontotemporal region one obtains "eating" automatisms; vocal and postural components of such states as attack, defense, and escape; and stances and movements associated with pause, alerting, and searching. Signs of autonomic activity, independent of or related to the foregoing behavior, also resulted from stimulation of widespread points and included lacrimation, salivation, pupillary effects, piloerection, cardiorespiratory changes, and, more rarely, urination and defecation. On occasion stimulation involving cortical structures appeared to have the effect of organizing stereotyped responses ordinarily elicited from this region into complex dynamic behavioral patterns. Of particular interest was the organized and ***directed*** angry defense

or attack that sometimes followed stimulations involving the pyriform cortex or the hippocampus near the amygdala.

Gastaut[21] has observed similar reactions in the cat.

In as yet unpublished experiments in monkeys, Delgado has shown that stimulation of the ventromedial quadrant of the frontal lobes, the anterior part of the cingulate gyrus, or the fornix, produces a similar picture—the ferocious male macaque may become completely docile at the moment that stimulation is applied but resumes ferocious behavior the moment that stimulation is stopped. This is not an arrest reaction, because the animals are able to move and to regain equilibrium if they are pushed; the effect seems to be connected with a decrease of aggressiveness.

FUNCTIONAL INTERRELATIONS BETWEEN LIMBIC CORTEX AND NEOCORTEX

With trained baboons, monkeys, and chimpanzees it has become possible through the use of specific tests (feasible for man as well as for animals) to investigate the effect of specific ablations of neo- and limbic cortex on learned skills and motor performance. Lesions restricted to the limbic system generally cause little or no impairment of intellectual functions such as visual discriminatory capacity, cutaneous or weight discrimination, whereas a lesion of the occipital neocortex causes gross impairment of visual discrimination, and parietal lobe ablation is followed by impairment of weight discrimination and cutaneous perception capacities. Lesions involving the frontal areas, such as those that occur in a radical lobotomy, impinge both on the frontal limbic system and on the frontal neocortex. Lesions of the frontal neocortex alone, when bilateral, impair intellectual functions as tested by the delayed-reaction response. Lesions restricted to the medial limbic structures, on the other hand, do not ordinarily affect visual discrimination and other types of learning known to be integrated by the neocortex; limbic lesions, in addition to affecting emotional behavior, may, however, impair some learned skills such as those involved in alternation testing.

Quite recently Delgado and Rosvold[10] have disclosed that in the monkey the disturbances in simple alternation, activity, and eating behavior usually attributed to the standard lobotomy may be obtained by electrical stimulation or electrocoagulation of small discrete areas in the ventromedial quadrant of the frontal lobe. This observation appears of very considerable importance because there is an increasing body of evidence[15, 23, 24] indicating that the beneficial effect of a frontal lobotomy in man can be obtained by limited section of the medial ventral quadrant involving primarily the connections of the limbic system and possibly also the head of the caudate

nucleus. Through the use of multiple electrodes in which stimulation or coagulation can be brought about at various levels within the frontal lobe substance of monkeys, individual regions can be studied; the behavior change just described is found only when medial ventral structures are

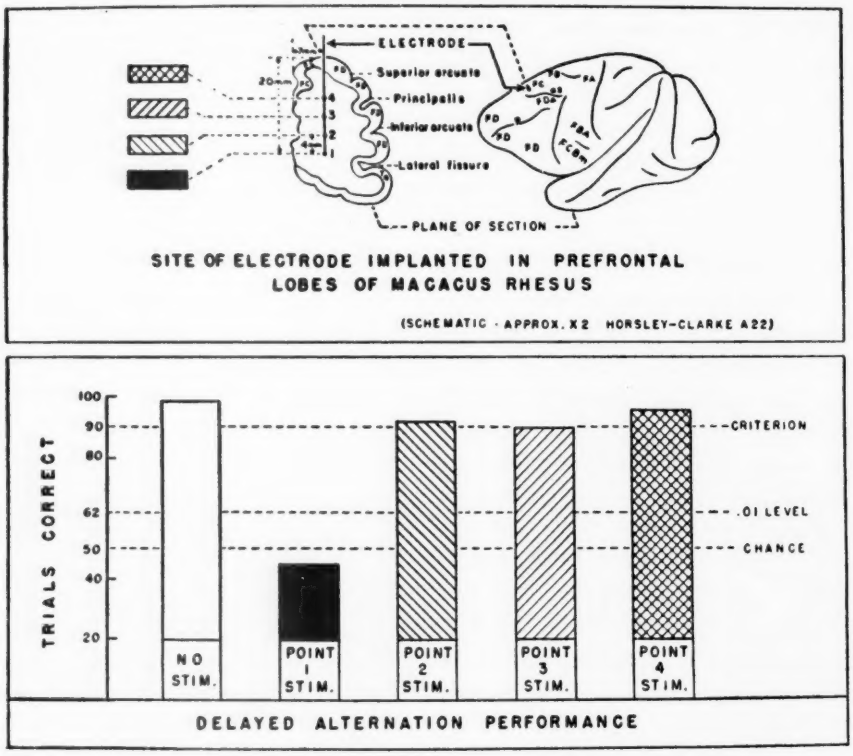

FIG. 5. (From Delgado and Rosvold, unpublished, see Ref. 10.)

involved. The accompanying diagram (Fig. 5) indicates these relations, and the following illustration shows the coagulated area that caused abolition of alternation skill in a monkey.

Applications in human subjects. Delgado and his associates[9] have now been able to apply these disclosures to the human subject suffering from intractable pain and they have found, as in the monkey, that coagulation of a corresponding area of the medial ventral quadrant has caused dramatic

relief—this in four cases (one of which has now been under observation for eighteen months with only minimal recurrence of pain). It is too soon to report on the other human cases, but initial results, as far as pain is concerned, have been striking and, even more important, these patients have shown none of the gross intellectual deficits which follow a radical lobotomy —this for the obvious reason that the neocortex had not been encroached upon in the coagulation procedure.

In patients suffering from severe pain a special pattern of electrical activity has been found, consisting of slow waves of high voltage localized in a small area of the frontal lobes. What is particularly interesting in this finding is that in one patient, immediately after the coagulation of this area, the slow waves disappeared and at the same time the pain was relieved. Of course it is not possible to generalize on the observation in one patient, but this offers an interesting possibility for future study.

In a patient with anxiety neurosis, startle behavior and searching, with apparent increase in anxiety, have been repeatedly evoked by electrical stimulation through electrodes implanted in the ventromedial quadrant of the frontal lobes.

Hence one can explore a human being suffering from pain by means of an electrode inserted through a small superior burr hole (guided by fluoroscope) ; when heightened electrical activity is encountered, the region can immediately be coagulated, and the patient, being conscious, can inform the surgeon when his pain has been relieved.

In summarizing our present concept of the interaction between limbic system and neocortex in determining behavior, I can do no better than to cite MacLean's colorful simile[32]:

> One might imagine that the neopallium and the limbic system function together and proceed through the world like a man on a horse. Both horse and man are very much alive to each other and to their environment, yet communication between them is limited. Both derive information and act upon it in a different way. At times the horse may shy or bolt for reasons at first inexplicable to his rider. But the sympathetic horseman will try to find out and understand what it is that causes the panic so he can avoid the disturbing situations in the future or reassure and train the beast to overcome them. One may think of psychotherapy as serving in a similar capacity, helping the intellectual faculties of the patient to ferret out the disturbing factors in his life situation so they can be dealt with and controlled in an intelligent fashion. In the case of the psychosomatic patient one suspects this helps to prevent excessive "neighing" along the streets [paths] of slow-moving traffic to the viscera.

COMMENT

Having long espoused the belief that experimental work on animals, and especially that carried out on higher forms such as monkeys and chimpan-

zees, is applicable in elucidating problems of function in man, it is particularly gratifying to be able to speak on lobotomy at Lisbon; for here it was that two courageous men, moved in some measure by the results of animal experimentation, but also moved equally and perhaps more wisely by significant clinical studies on the frontal lobes, put the matter to test by introducing the leucotomy procedure. In 1935 at the Second International Neurological Congress in London, Carlyle Jacobsen and I reported[18,8] that two rather emotional chimpanzees exhibited interesting behavioral changes following bilateral frontal ablation; their manifestations of anxiety in test situations and their emotional outbursts ("temper tantrums") in the face of frustration largely disappeared following the bilateral operation. We were not aware then of the distinction between the limbic system and the neocortex, but histological study of the brains of these two animals (Becky and Lucy) indicates that both neo- and limbic cortex had been ablated; in retrospect it is also significant that the animals became rather "stupid" after the procedure and showed recognizable but not gross impairment of various forms of learned behavior.

Jacobsen and I had not suggested that our chimpanzee results might point to a valuable therapeutic tool for dealing with mental disease. This stemmed entirely from the creative genius of Egas Moniz, aided by the talent and skill of his gifted colleague, Almeida Lima. For this and many other reasons we now salute them "for their imagination, perseverance and daring."

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A COMPARATIVE STUDY OF THE ALKALI RESERVE OF NORMAL, PREGNANT, AND FETAL SHEEP*

In a study of the carbon dioxide exchange between the fetal and maternal bloods of goats, Keys[3] observed that the alkali reserve of the fetal blood sampled after caesarian section was higher than that of the maternal plasma obtained after the delivery of the fetus. The alkali reserve of the fetal kids fell within the range characteristic of normal barren goats, but that of the mother was in every case below the normal range. These observations, made on goats in the last 30 days of the gestation period, led him to infer that the pregnant goat is in a state of metabolic acidosis, an inference that gained support from studies by Barcroft and his collaborators[1] indicating that the position of the oxygen dissociation curve, prepared at constant CO_2 pressure with the blood of gravid goats, was to the right of the field characteristic for the curves similarly prepared with blood from individuals not gravid.

My own observations on the carbon dioxide content of fetal and maternal plasmas of sheep,[2] drawn under similar circumstances after the lambs had been delivered by caesarian section, indicated that here, too, the concentration was higher in the fetal plasma. This observation, viewed in the light of the results of Keys[3] and Barcroft *et al.*,[2] pointed to the possibility that the gravid ewe like the goat was in a state of metabolic acidosis. Hence, I determined to examine this possibility in the hope of learning something about the genesis and development of pregnancy acidosis. The results obtained form the substance of this report which deals with the alkali reserve in normal barren ewes, in gravid individuals at several stages in gestation, and fetuses in a number of different stages of development.

As the first step in the investigation the alkali reserve was determined in a series of normal ewes that were not pregnant and had not recently been so, in an effort to establish the level and the range of variation characteristic of individuals kept under usual farm conditions. Those objectives obtained, I next followed the changes in the alkali reserve in a small series of ewes, with appropriate controls, before, during, and after the gestation period.

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Inasmuch as the results of the second step in the investigation indicated that the alkali reserve of the blood of the pregnant ewes, as sampled, did not fall outside the range of variation of the controls, I was obliged to consider the possibility that the lower carbon dioxide content of the maternal blood at delivery by caesarian section might be due—in my earlier cases and in those of Keys[3] and Barcroft *et al.*[2] as well—to some circumstance associated with the preparation for and/or the delivery of the fetus by caesarian section.

The testing of this possibility formed the third stage in this program of study. Comparisons were made between the alkali reserves of plasmas obtained from blood samples drawn before and after preparation for the laparotomy with spinal or nembutal anesthesia. The results indicated quite clearly that the alkali reserve of the ewe was lowered by the procedures antecedent to the laparotomy—the administration of the anesthetic and placement on the back on the operating table. Clearly the final stage, the comparison of the alkali reserve of the fetus and the mother, could not be considered representative of circumstances normally occurring *in utero,* except in those cases in which the reserve of the mother was not substantially altered by the procedures associated with the delivery of the fetus. When the ewes for this part of the study were chosen for their quiet temperament and the fetus was delivered after the application of a local anesthetic to the abdominal wall, with the ewe lying on her side, her head gently supported by an attendent, I succeeded in obtaining samples for comparison in which the maternal alkali reserve was not altered more than three volumes per cent from the time the first sample was drawn in the pen until the end of the experiment. The data so obtained I have taken to be representative of normal circumstances *in utero.*

MATERIALS AND METHODS

For these studies, as in my earlier ones, I have used good grade or registered Dorset and Shropshire ewes; they were kept by Dr. and Mrs. Morton Loeb on their Bethany farm with their own flock. The Loebs provided a record of the breeding dates indispensable for the investigation. The ewes had the run of a good pasture with a daily ration of grain and alfalfa hay *ad libitum* during the winter months. An open barn provided shelter in foul weather.

The blood samples in these studies were drawn into an oiled syringe containing a solution of potassium oxalate and ammonium fluoride (2% $K_2C_2O_4$ and 0.6% NH_4F) in an amount such that the final mixture contained nine parts of blood and one of the anticoagulant; the results were corrected for this dilution. The alkali reserve of the true plasma was determined in each case from the carbon dioxide dissociation curves prepared in the usual manner by equilibrating oxygenated blood samples in tonometers filled with air to which varying amounts of CO_2 were added. The number of points

determined depended upon the size of the blood sample available, but was never more than five nor less than two. If only two points were to be determined the CO_2 pressures in the tonometers were adjusted to about 35 and 50 mm. Hg. respectively. Equilibration was carried out in a water bath at 39° C.; after ten minutes the gas pressure was brought to atmospheric by opening the stopcock of the tonometer with its tip just below the surface of the water in the bath and allowing the excess to escape. At the end of the equilibration period—20 minutes—the blood was removed into a syringe, lubricated with mineral oil, by driving the needle through the rubber stopper that closed the other end of the tonometer. The larger fraction of the sample was transferred to a centrifuge tube filled with oil and closed by a one-hole stopper; as the blood entered the tube, the oil escaped through the hole which was closed with a glass plug when only a thin layer of the oil remained to cover the blood. Cells and plasma were separated by centrifugation.

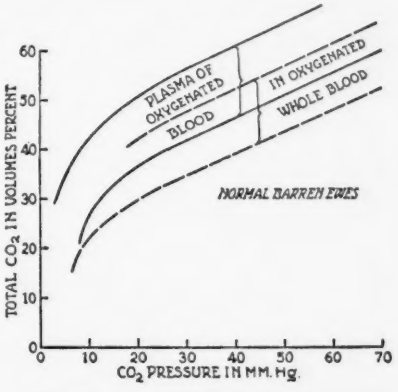

FIG. 1. A graph indicating the range of variation in the carbon dioxide dissociation curves — oxygenated whole blood and true plasma — in a series of ewes not pregnant or recently so.

The carbon dioxide content of the whole blood was determined by Van Slykes' manometric method on 0.5 cc. samples transferred directly from the syringe to the pipette. Determinations were similarly made on the plasma drawn from the centrifuge tube and transferred to the pipette by means of an oiled syringe. The concentration of CO_2 in the tonometers was determined with the Henderson-Haldane apparatus.

RESULTS

The alkali reserve in normal ewes. For the initial observations on the alkali reserve of normal ewes twelve mature individuals were available. The blood samples were drawn from the external jugular vein whilst the ewes were standing in a small pen to which they had been confined an hour or two earlier. The results obtained on the blood samples so drawn were used to construct Figure 1; the solid lines represent the field within which the points obtained from the analysis of the true plasma fell. The points obtained with whole blood fell within the area limited by the dotted lines. Taken together these data indicate that the alkali reserves of this series of ewes ranged between 46.5 and 61.0 volumes per cent—a range that would appear to be fairly representative of the normal variation to be found in ewes kept under the usual farm conditions.

The alkali reserve in pregnant ewes. Ten ewes were used in this study in which the alkali reserve was followed in each individual at approximately

tri-weekly intervals over a period of several months during which eight of them were bred and gave birth to healthy lambs; the two that were not bred served as controls. Two of the ewes bore twins, the remaining six, singlets.

The blood samples for the preparation of the CO_2 dissociation curves were drawn from the jugular vein with the ewe standing quietly in the small pen to which she had been confined for an hour or so. With but two exceptions the alkali reserve in these ewes never varied, in the period they were under study, beyond the limits described above as characteristic of the normal, non-gravid animals. The data obtained on these two exceptions —one bore twins, the other a singlet—are illustrated graphically in Figure 2, together with the data on one of the controls; they indicate that the alkali reserve of these two ewes never fell below the range found in normal ewes, but did rise above that range shortly after insemination. The significance of this rise, if it has any, is not clear, nor is that of the fall that follows in the winter months, but the latter is clearly not due to the gravid state of the ewes for it occurred in the controls as well.

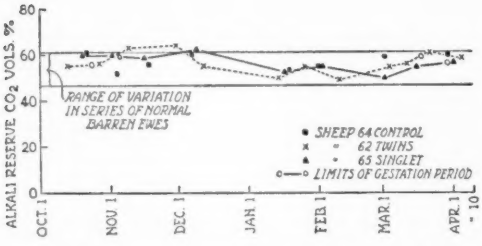

FIG. 2. A graph representing the variation in the alkali reserve of three of a series of ewes followed over months and including the gestation period.

The effects of handling and anesthetics on the alkali reserve. As these observations on the alkali reserve of pregnant ewes lent no support to the view that they were acidotic when the blood samples were drawn while they were standing quietly, the possibility that the low bicarbonate levels of the plasmas of goats and sheep when sampled at caesarian section was due to circumstances associated with the preparation for the operation appeared worthy of investigation. The experiments were carried out as follows: A first sample of blood was drawn from the external jugular vein while the ewe was standing quietly in her pen; the spinal anesthetic or intravenous nembutal was then given and the ewe placed on her side or back on the operating table and restrained as in preparation for the caesarian section. At selected intervals thereafter blood samples were drawn from the jugular for the preparation of carbon dioxide dissociation curves of oxygenated blood and true plasma as mentioned above. Six ewes were studied in this manner. In each case the alkali reserve of the plasma as sampled fifteen to twenty minutes after the ewe was anesthetized and restrained was between

2.5 and 10.7 volumes per cent less than that of the first plasma sample; the fall in the alkali reserve is illustrated in Figure 3. These curves were prepared with the blood of sheep 12, and the second sample was drawn twenty-two minutes after the ewe had been anesthetized with nembutal and placed on her back. In another ewe given a spinal anesthetic and placed on her side the alkali reserve fell from 52.5 to 44.1 volumes per cent in the interval of an hour. The details are given in Table 1.

These observations demonstrate the influence of the anesthetic and handling of the ewes on their alkali reserve; further, they indicate quite clearly that inferences drawn about the relative levels of the fetal and maternal alkali reserve in pregnant sheep on data obtained from samples drawn at caesarian section may not represent the circumstances as they exist in an individual undisturbed in her pen or grazing in a pasture.

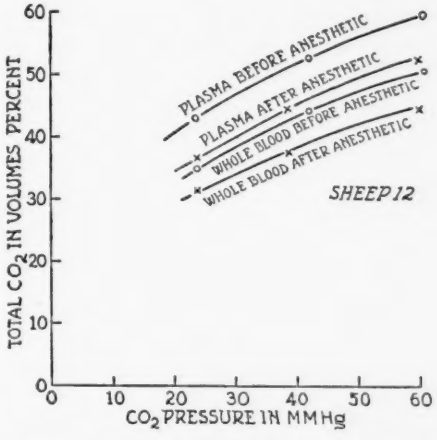

FIG. 3. The carbon dioxide dissociation curves — oxygenated blood and true plasma — prepared with samples from the jugular vein before and after administration of intravenous nembutal.

Clearly, in order to establish the relative levels of the alkali reserve of fetal and maternal plasmas in sheep as they exist at several stages of pregnancy the blood samples must be obtained under conditions in which the bicarbonate levels are not altered from normal. In the course of my efforts to obtain such samples, I assumed that circumstances that failed to alter the maternal plasma levels would be without appreciable effect on the fetal. After a number of unsuccessful trials with general anesthetics, I finally hit on a procedure by which the abdomen of the ewe could be opened, the fetus delivered, and a blood sample drawn without altering her alkali reserve more than three volumes per cent in the interval. In some cases there was no change.

The procedure was as follows: I selected a series of eight ewes that had been handled a good deal and were of a placid nature. They were quite accustomed to lying on their sides on a table with their heads cradled by an assistant and without anything to restrain their movements. The anterior abdominal wall was anesthetized with a local anesthetic, given through a new and sharp no. 20 needle. After a blood sample had been drawn from

the external jugular, the abdomen was then opened with a minimum of delay, a single incision separating all the layers and exposing the uterus. Again a single incision exposed the fetal vessels, the fetal sample was drawn, and the second sample obtained from the maternal jugular in an interval of five minutes.

TABLE 1. CHANGES IN ALKALI RESERVE FOLLOWING ADMINISTRATION OF SPINAL ANESTHETIC AND PREPARATION FOR LAPAROTOMY

Time	*Position of ewe*	*Alkali reserve vols. %*
10:45 a.m.	Standing	52.5
11:00 a.m.	Given spinal—placed on side	49.
11:20 a.m.	Lying on side	48.8
11:45 a.m.	Lying on side	44.1

TABLE 2. THE ALKALI RESERVE OF PREGNANT EWES AND THEIR FETUSES

Sheep no.	*Fetal age*	*Fetal alkali reserve*	*pH at pCO_2 40 mm. (calculated)*	*Maternal alkali reserve*	*pH at pCO_2 40 mm. (calculated)*
54	62	47.5	7.32	57.3	7.41
8	81	44.7	7.29	54.5	7.38
2 gr	109	48.5	7.33	55.5	7.39
50	111	56.5	7.40	57.0	7.40
52	116	56.2	7.40	56.7	7.40
70	126	48.2	7.33	49.6	7.34
3	136	37.9	7.22	37.8	7.22
60	140	58.3	7.42	62.0	7.44

The results of these experiments are presented in Table 2. The maternal alkali reserve given is that determined on the first sample of blood drawn from the external jugular whilst the ewe was standing. The actual dissociation curves of two cases, No. 54 and 52, are illustrated in Figures 4 and 5. If these data represent the normal relations *in utero,* they indicate that the fetal alkali reserve is about equal to or less than that of the maternal blood. In no case was it higher. They further indicate that the fetal reserve

is lower in early stages of gestation—in the period of implantation—relative to the maternal than it is in the last month of gestation though the data would not warrant a final conclusion.

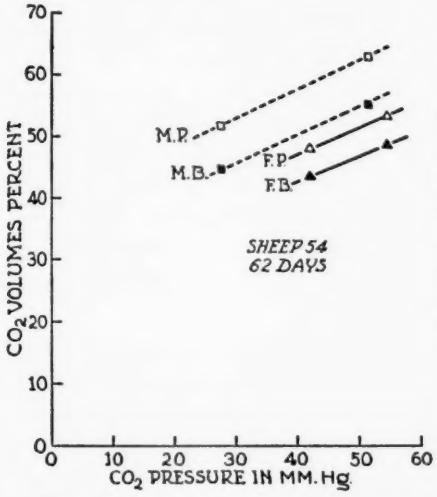

FIG. 4. Fetal and maternal carbon dioxide dissociation curves of oxygenated whole blood and true plasma. Sheep No. 54, 62 days.

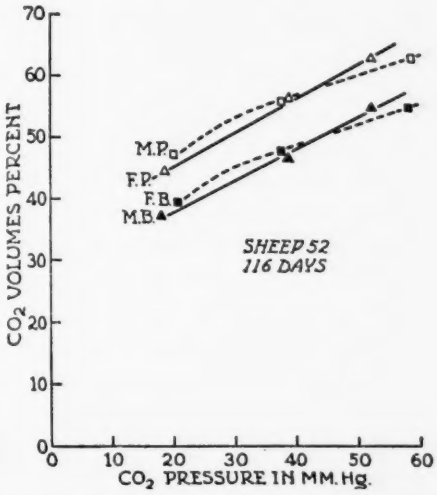

FIG. 5. Fetal and maternal carbon dioxide dissociation curves of oxygenated whole blood and true plasma. Sheep No. 52, 116 days.

SUMMARY AND CONCLUSIONS

The observations presented here appear to justify two conclusions: (1) that the alkali reserve of the ewe is not diminished during pregnancy below the range established for normal non-gravid individuals, and (2) the reserve of the fetal plasma is equal to or less than that of the ewe. These results suggest that the plasma of the sheep is not affected by pregnancy in the same fashion as reported by Keys in the goat in which the maternal alkali reserve appears to be lower with respect to that of non-gravid animals and fetal kids. Where these differences in the response of the pregnant sheep and goat have their origin is a matter of speculation and a subject for future research.

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THE EFFECT OF FASTING ON THE NUCLEIC ACID CONTENT OF THE TISSUES IN THE NORMAL AND ADRENALECTOMIZED RAT*

The adrenalectomized rat may be maintained on saline without significant changes in carbohydrate or protein metabolism. When such animals are fasted, however, hypoglycemia and depletion of liver glycogen rapidly occur, accompanied by a reduction in the amount of nitrogen lost in the urine. It has therefore been suggested that the fasting adrenalectomized animal is unable to mobilize body protein adequately for the maintenance of blood glucose and glycogen.[12]

Some change in nucleic acid metabolism might well accompany those changes which occur in protein metabolism in the fasting adrenalectomized animal. There is evidence that an intimate association between nucleic acid levels and protein synthesis may exist. The details of this relationship remain obscure, but the work of Caspersson[5] and of Brachet[2] indicates that ribonucleic acid (RNA) plays a particularly important part.

The effect of fasting on the RNA content of the liver in normal rats has been investigated by Davidson[6, 7, 8] and Kosterlitz[8] who found a fall in RNA during the fasting or in animals on low protein diets. The adrenalectomized animal's altered nitrogen metabolism and the known correlation of RNA levels with the state of protein metabolism, then, suggest that some further change in nucleic acid content might be detected in the fasted animal whose adrenals have been removed.

METHODS

The method of Schneider[17, 18] was used for the analysis of RNA and desoxyribonucleic acid (DNA) in liver, kidney, brain, and muscle from adult rats. The following improvements were introduced:

1. Tissues were obtained under nembutal anaesthesia rather than after death from a blow on the head.

2. Dry ice was used to keep tissue samples frozen or as cold as possible during preparation and homogenization.

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RESULTS

The tissues of the first two animals of the series were treated as suggested by Schneider yielding 80 ± 0.5 mg. RNA per 100 gm. of liver. When dry ice was substituted for cracked ice, 98 ± 3.2 mg. (seven animals) was obtained. Dry ice was therefore employed in all subsequent experiments. RNA content in frozen samples was unchanged up to four days after autopsy.

The effects of fasting and adrenalectomy on nucleic acid concentrations in the liver are summarized in Table 1. Adrenalectomy did not affect DNA

TABLE 1. THE EFFECT OF FASTING ON LIVER NUCLEIC ACID CONCENTRATIONS IN NORMAL AND ADRENALECTOMIZED RATS

	Control *mg./100 gm. wet weight*	*Adrenalectomized* *mg./100 gm. wet weight*
DNA		
Fed	40 ± 1.7 (8)	40 ± 2.3* (5)**
Fasted 24 hrs.	47 ± 1.2 (7)	51 ± 1.3 (10)
Fasted 12 hrs.	40 ± 3.2 (5)	44 ± 3.8 (5)
RNA		
Fed	98 ± 3.2 (7)	106 ± 4.0 (5)
Fasted 12 hrs.	95 ± 3.3 (5)	100 ± 3.4 (5)
Fasted 24 hrs.	101 ± 3.2 (7)	97 ± 2.9 (7)
Change in liver volume	15%	21%
Fasted 24 hrs. corrected for change in liver volume	86 ± 2.6 (7)	77 ± 2.1 (7)

* Standard error of the mean.
** Number of animals.

concentrations in fed rats. Statistically significant elevation of DNA concentration during 24-hour fasts occurred, however, in both control and adrenalectomized animals. The rise was significantly greater in the adrenalectomized than in the control group.

For reasons to be discussed, the values in Table 1 are corrected to represent liver RNA concentrations in fasting animals per unit of original tissue before fasting. In spite of the unchanged RNA concentration in fasting normal and adrenalectomized animals, total liver RNA decreased in both groups and a larger fall occurred in the adrenalectomized series.

Nucleic acid determination in the normal kidney revealed a DNA of 55 mg. and RNA of 54 mg. per 100 gm. wet weight. There were no significant changes in these normal levels after fasting or adrenalectomy.

Similar studies were attempted on brain and muscle but were abandoned when it appeared that changes, if any, would probably not be of a magnitude demonstrable by the method.

DISCUSSION

METHOD

Much work has been done with both the Schneider and Schmidt-Thannhauser[18] methods of nucleic acid determination. Schneider's simpler method was chosen because it was not necessary to separate RNA from DNA.

Schneider obtained tissues by killing the animals with a blow on the head and placing the samples on cracked ice. By this procedure he demonstrated about 55-71 mg. RNA per 100 gm. of liver in the normal rat.[19] Our early values were higher than his, and it was thought that this difference might be attributable to the precautions which were taken to keep the tissues cold during handling. Freezing was therefore introduced, and a still higher yield of RNA was obtained. Davidson[8] has summarized the results reported by other authors who used the Schmidt-Thannhauser and Schneider methods. Our values are for the most part higher in all tissues than those obtained by others using the Schneider method. Greater yields of nucleic acids probably result from the use of frozen tissue and from the analysis of samples taken from an anaesthetized animal rather than a dead one. These observations indicate that nucleic acids are unstable and tissues to be analyzed should be treated with as much speed and kept as cold as possible. The consistency of the lower values obtained by us and by the other authors using the Schneider method without freezing suggests the possibility of two kinds of RNA, one of which is more labile than the other.

Although it is usually stated that the Schneider and Schmidt-Thannhauser methods yield the same amounts of nucleic acids, Davidson's review shows that authors using the Schmidt-Thannhauser procedure obtained slightly higher yields than those using Schneider's. Values for nucleic acids of the liver, kidney, and brain in the present study exceeded slightly or corresponded with the higher limits which others have observed using the Schmidt-Thannhauser method. It should be noted here that the analysis of muscle has not been attempted by very many investigators and that results

have varied widely for both RNA and DNA. RNA, for example, is 2-10 mg. per 100 gm. according to Schneider and Klug,[19] 22.8 mg. per 100 gm. according to Mandel[13] and 13 mg. per 100 gm. by our techniques.

THE EFFECT OF FASTING AND ADRENALECTOMY ON NUCLEIC ACIDS IN THE LIVER

Fasting. It has been shown[3, 4, 6] that total DNA in the liver does not change during fasting. An increase in DNA concentration, therefore, is a measure of the reduction in liver size during fasting. From the data obtained, it was calculated that the normal animals lost 15 per cent and the adrenalectomized 21 per cent of their liver weight during a 24-hour fast (Fig. 1). Using this information, the changes in total RNA under similar circumstances may be calculated. Calculations (Table 1) showed that a significant decrease in RNA occurred on fasting which is in agreement with the conclusions of Davidson and Kosterlitz.

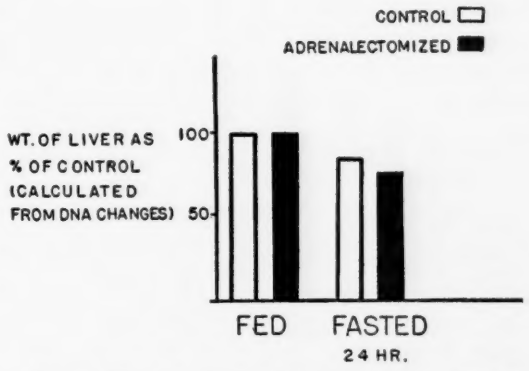

FIG. 1. The effect of fasting on liver weight in normal and adrenalectomized rats, calculated on the basis of the change in DNA concentration.

Adrenalectomy. Similarly, calculated values of RNA in the adrenalectomized animals fell on fasting and to a significantly greater degree than in the controls.

The possible influence of the endocrine glands on nucleic acid metabolism is indicated by the variations in nucleic acid levels which have been reported in animals of different ages, growth, and nutritional state. RNA in the liver is said to increase in pregnancy,[4] while estradiol treatment of pigeons results in a rise of DNA and a large increase in RNA and phosphoprotein in the liver.[14] Hypophysectomy causes a reduction in the rate of radioactive phosphorous turnover in the thymus and liver according to Fraenkel-Conrat,[9] and Geschwind has been able to demonstrate a fall in liver RNA after hypophysectomy. Normal levels are restored by growth hormone.[10] Baker has reported a reduction in liver RNA in rats treated with adrenocorticotrophic hormone and a high carbohydrate diet.[1]

Histological changes in the liver after adrenalectomy have been described in white mice by Müller. Among other changes, he has observed an increase

in both the size of the nuclei and the number of binucleated cells.[15] Gros has studied the effect of large doses of cortisone acetate on the livers of rabbits.[11] He finds a hypertrophy of the liver accompanied by a decrease in DNA and no effect on RNA, expressed in mg. per 100 total protein, which suggests that changes in RNA, if any, are paralleled by those in total protein of the liver, and raises the question whether the increased nitrogen excretion of the cortisone-treated animal is accompanied by an increase of RNA in mg. per 100 gm. wet weight.

The significance of the greater fall in RNA in the liver of the adrenalectomized fasting rat as compared to the normal rat is obscure in view of the many functions attributed to the nucleic acids. Nevertheless, the intimate association of RNA with protein synthesis and the apparent reduction of protein catabolism in the adrenalectomized animal implicates the adrenal cortex either in a direct action on RNA or an indirect one in which protein itself is the target substance.

SUMMARY

1. The use of anesthesia instead of decapitation and the freezing of tissues during preparation significantly increased the yield of nucleic acids. The possibility of a labile fraction of RNA is suggested.

2. Adrenalectomy did not affect nucleic acid levels in the tissues studied in fed rats.

3. Total RNA in the liver decreased significantly on fasting for 24 hours and underwent a significantly greater reduction in the fasting adrenalectomized animals.

4. No significant changes in RNA or DNA were demonstrated in kidney, brain, or muscle tissue after adrenalectomy and fasting.

ACKNOWLEDGMENT

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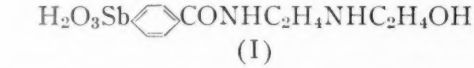
A CHEMOTHERAPEUTIC STUDY OF ANTIMONIALS IN THE EXPERIMENTAL INFECTION OF MICE WITH CRYPTOCOCCUS NEOFORMANS

The use of antimony compounds, particularly potassium antimonyl tartrate (Tartar emetic), in Cryptococcosis has occasionally been mentioned in the literature,[2, 5] but no experimental study of the therapeutic rationale of the use of antimonials in this infection has to our knowledge ever been undertaken. It did not seem that Tartar emetic was of any therapeutic value, but this should not reflect on the chemotherapeutic possibilities of the entire class of antimony derivatives. In rather extensive investigations on the therapeutic sensitivity of the chronic infection of mice with *C. neoformans* carried out in this laboratory it was observed that certain antimonials do indeed possess a marked effect in this otherwise resistant infection.

The active antimonials belong to the group of carbamylbenzene stibonic acids, synthesized by Dr. N. Steiger[8] of the Roche Chemical Research Laboratories. The most active member of this series was (4-[β-β-hydroxyethylamino)ethylcarbamyl] benzene stibonic acid (I), designated Ro 2-3094.



(I)

The compound contains approximately 31% Sb and was used in the form of its water soluble diethanolamine salt.

MATERIALS AND METHODS

Strain of C. neoformans. This strain was received through the courtesy of Dr. M. Tager, Emory University, Atlanta, Georgia in 1947 and has been kept on glucose Sabouraud agar slants ever since. Transfers were made as a rule every 2d or 3d day. For the infection of mice 48-hour cultures in Sabouraud's broth were employed.

EXPERIMENTAL PROCEDURE

Mice of 18-22 g. from one colony were used in these experiments. The animals were infected intra-abdominally with 0.5 ml. of a 48-hours *C. neoformans* broth culture corresponding to 1,000,000 to 2,000,000 viable organ-

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isms according to plate counts. This infection was fatal for an average of 80% of the animals within three weeks, with the majority of the animals dying between the 10th and 14th day. The infected mice were divided into groups of 10 to 20 animals. The treatment, always by the subcutaneous route, was started within 5-10 minutes after the infection. In most experiments one daily dose was given for 21 days. All animals that died at least five days after the infection were autopsied, the organs were examined, and cultures on Sabouraud agar taken which were read after 48 hours' incubation. On the 22d day all surviving animals were sacrificed, examined, and cultured in the same way. The pathological lesions produced by the infection used were most marked in the lungs. Liver and spleen showed little or no gross involvement. The lungs showed a high degree of pathological change in the form of numerous whitish nodules which tended to become confluent. Therefore, the criteria on which evaluation of Ro 2-3094 and other comparative compounds was based were the gross lesions of the lungs and the cultures derived from this organ. In several instances cultures were also taken from the brain. Since the results of the brain cultures were almost identical with those of the lungs, brain cultures were not taken regularly.

The extent of gross lung lesions and of the growth on agar slants was estimated using the symbols 1 + to 3 +. In this type of grading 3 + indicated widespread lesions in all pulmonary lobes and—in cultures—the complete covering of the agar surface with confluent growth; 2 + and + signify lower degrees of involvement and lighter growth of cultures. In cases where very few and small lesions and not more than 20 colonies were seen, actual counts were made and, for the purpose of averaging, designated as 0.5 + or less. Absence of the lesions and growth on agar was marked as zero. The average of the lesions and cultures was calculated from the sum of the plus and minus symbols.

This evaluation was supplemented by the ratio c/t of average of lesions in controls: average of lesions in treated animals, and the same ratio was calculated from the results of the cultures. Inactive compounds had a ratio close to 1.0. Activity was characterized by values of more than 2.0.

EXPERIMENTAL

TOXICITY OF RO 2-3094

The acute toxicity was determined by single injection into mice using the subcutaneous and intravenous routes and in rabbits by intramuscular or intravenous injection. The observation time was at least three days. The results as given in Table 1 indicate that the compound showed the comparatively low toxicity characteristic for stibonic acids.

Repeated daily drug administration of 50, 100, and 250 mg./kg. was well tolerated by the majority of mice for periods up to three weeks. Rabbits tolerated 10 intramuscular injections of 20 mg./kg. given alternately into the right and left hind leg without symptoms of systemic toxic effects. However, the animals showed at autopsy the characteristic tissue necrosis which almost invariably follows the injection of antimonials.

TABLE 1. ACUTE TOXICITY OF SINGLE DOSES OF RO 2-3094

Animal	LD_{50}: *mg./kg.*[a] *subcut.*	*intramusc.*	*intraven.*
Mouse	591.0	...	350.0
Rabbit	....	90.0	23.0

[a] The figures refer to the free stibonic acid.

TABLE 2. EFFECT OF ANTIMONIALS IN THE C. NEOFORMANS INFECTION OF MICE

Infection: approx. 1.5 x 10^6 viable cells
Therapy: subcut. injection daily for 21 days
Autopsy: 22d day

Compound	*Dose: mg./kg.* *comp'd*	*Sb.*	*Survival rate*[a]	*Average* *lesions*	*culture*	*Ratio c/t*[b] *lesions*	*culture*
T. emetic	5.0	1.83	0/10	2.5	2.9	0.96	0.96
Fuadin	37.5	5.10	2/10	2.2	2.7	1.09	1.03
Neostibosan	50.0	20.0	0/10	1.9	2.4	1.26	1.16
Ro 2-1160	100.0	36.0	0/10	2.3	2.5	1.04	1.12
Ro 2-3094	50.0	15.5	23/30	0.57	0.3	4.21	9.3
Controls	....	....	4/44	2.4	2.8	...	...

[a] No. surviving: no. observed mice.
[b] Lesions in controls: lesions in treated mice.
Cultures in controls: cultures in treated mice.

ACTIVITY AGAINST C. NEOFORMANS

(a) *In vitro.* A survey of the literature showed clearly that in the case of *C. neoformans* there is a very unsatisfactory correlation of activity *in vitro,* which is frequently very high, and *in vivo,* where chemotherapeutic effects are generally absent. Ro 2-3094, though not selected on the basis of *in vitro* activity, possesses only a modest growth-inhibiting property. In the usual dilution test in Sabouraud's glucose broth using 0.05 ml. of a 48-hour broth culture as inoculum, the fungistatic concentration was found to be 0.5 mg./ml., a result which was in fair agreement with the value of 1.5 mg./ml.

recently published by Carton and Liebig.[8] Addition of 5% goat serum did not interfere with the activity in our experiments.

(b) *In vivo.* The stibonic acid Ro 2-3094 was compared with a group of known antimonials, namely: potassium antimonyl tartrate (Tartar emetic), antimony-pyrocatechol sodium disulfonate (Fuadin), as representatives of tervalent Sb compounds, and with the pentavalent compounds: Neostibosan is a mixture of the diethylamine salts of p-aminophenyl stibonic acid, p-acetylaminophenyl stibonic acid and antimonic acid; Ro 2-1160 is a combination of 2-carboxymethylmercaptobenzene stibonic acid and calcium salt.[7]

TABLE 3. SYNOPSIS OF 16 EXPERIMENTS WITH RO 2-3094 IN THE C. NEOFORMANS INFECTION OF MICE

Infection: 1 x 10^6 to 2 x 10^6 viable cells
Therapy: daily subcut. injection for 21 days; dose 50 mg./kg./day

	Survival		*Average*		*Ratio c/t*	
Group	*rate*[a]	*%*	*lesions*	*culture*	*lesions*	*culture*
Treated	130/170	77.2	0.66	0.58	3.18	4.66
Controls	22/108	20.4	2.10	2.7	...	...

[a] No. surviving mice: no. observed mice.

These compounds, as well as Ro 2-3094, were given at a dose of approximately one-fifth of the tolerated dose as found at single administration.

The results as given in Table 2 show that only Ro 2-3094 exerted a marked effect in the *C. neoformans* infection. This is shown by the high survival rate and by the appreciable reduction in the extent of lung lesions and of organisms recovered by culture as compared to controls and unsuccessfully treated mice. As a rule 50% or more of the animals were free of lesions and gave negative cultures. This effect appeared to be independent of the Sb content, if one compares the three stibonic acids which were given in comparable doses. The tervalent antimonials cannot be used for such a comparison.

It might also be mentioned that 15 additional antimonials, most of them stibonic acids, have been tested, but only one of them, closely related to Ro 2-3094, showed activity against *C. neoformans* and this in lesser degree.

The experiments with Ro 2-3094 have been repeated on different occasions, altogether 16 times, and the same results were obtained with negligible variations. A survey of these experiments is given in Table 3.

The animals listed in this table also contain a group of 40 treated and 27 untreated mice in which cultures were taken from the brain. The results were very similar to those obtained with the lungs. The average extent of growth of the brain cultures from the untreated controls was 1.6, the corresponding value from the brain of the treated mice, 0.6. This gave a ratio c/t of 2.7. That this value is lower than that obtained in the evaluation of the lungs is due to the fact that average strength of the brain cultures was as a rule lower than that of the lungs. In an additional series of experiments we tried to increase the protective effect of Ro 2-3094 by increasing the dose

TABLE 4. INFLUENCE OF DIFFERENT DOSAGE SCHEDULES ON THE EFFECT OF Ro 2-3094 IN THE C. NEOFORMANS INFECTION OF MICE

Infection: 1 x 10^6 to 1.5 x 10^6 viable cells
A: 250 mg./kg. daily for 21 days
B: 100 mg./kg. daily for 21 days
C: 250 mg./kg. 2 times a week for 3 weeks
D: 50 mg./kg. daily for 21 days
Autopsy: 22d day

Dosage schedule	*Total dose: g./kg.*	*Survival rate*[a]	*Average of*		*Ratio c/t*	
			lesions	*cultures*	*lesions*	*cultures*
A	5.2	8/10	0.2	0.1	11.5	28.0
B	2.1	8/10	0.28	0.73	8.2	4.0
C	1.5	6/10	0.37	0.9	6.2	3.1
D	1.05	15/20	0.52	0.85	4.4	3.3
Controls	...	0/18	2.3	2.8	...	...

[a] No. surviving mice: no. observed mice.

of the drug. At the same time we attempted to reduce the frequency of administration by giving higher doses, e.g. the tolerated dose, of the drug. The results of this experiment in which four different dosage schedules were used are compiled in Table 4.

It will be seen from this table that all four dosage schedules, covering total doses of 5.2 to 1.05 g./kg., exerted the expected effect on the survival rate which varied from 60% to 80%, while all untreated controls died. Slight, statistically insignificant differences were observed in the extent of lesions. The reduction of culture growth was significantly greater in group A (total dose 5.2 g./kg.) than in the remaining groups treated with the lower doses of 2.1, 1.5, and 1.05 g./kg. The "P" values were less than 0.02 for the difference between groups A and B and less than 0.01 for the differences between

A and C and A and D. This seemed to indicate that at least the influence of the drug on the recovery of the organisms from the lungs was dependent on the dose within the range of dosages tested.

In one experiment in which the observation time was extended another three weeks after discontinuance of therapy (50 mg./kg. for 21 days) delayed death occurred in the group of treated mice. Nine out of 10 mice lived 27 days post-infection; seven of the mice died between the 30th and 40th day. Autopsy showed, however, that the extent of lung lesions and the growth on agar had not increased as demonstrated by the average values of 0.6 for lesions and 1.0 for cultures. The corresponding figures for the controls which had died within the first three weeks after the infections were 2.3 and 2.8, respectively. At present this observation cannot be fully discussed because we have not done Ro 2-3094 chronic toxicity experiments on non-infected mice. Practically identical findings can be found in one of the experiments with Fungicidin as reported by Hazen and Brown.[4]

Ro 2-3094 has not been tested so far in other fungus infections. The only other chemotherapeutic property we found was a slight trypanocidal activity in T. brucei infections which could, however, only be observed with the dose of 500 mg./kg. which was toxic for half of the treated animals.

DISCUSSION

From the data of the experimental part, we have concluded that the stibonic acid Ro 2-3094 under the conditions of the test exerts a marked effect on survival time, pathology, and cultural recovery of the causative agent in infections of mice with the strain of *C. neoformans* used in these experiments. Only one closely related stibonic acid had a similar although less consistent activity, while 18 additional organic antimonials and a great number of synthetic compounds from different chemical classes failed to show an influence in this infection. Ro 2-3094 seems, as far as can be learned from the literature, the only synthetic product able to control the *C. neoformans* infection, although sulfadiazine has been mentioned by Reilly and Artman[6] as prolonging life in infected rats. Antibiotics of microbial origin seemed more frequently to be efficacious in this fungus infection. Streptomycin[1, 6] and penicillin[6] had some activity in experimental infections, while Actidione,[2, 5] Polymyxin B,[3] and other antibiotics[5] were without effect in mice, although some of them, e.g. Actidione and Polymyxin B, may be potentially useful in central nervous system cryptococcosis of humans. High *in vivo* activity in cryptococcal infections is apparently limited to Fungicidin. Effects very similar to those observed with Ro 2-3094 and with occasionally longer lasting success were observed by Hazen and Brown.[4]

SUMMARY

The stibonic acid Ro 2-3094, a member of the group of substituted carbamylbenzene stibonic acids, exerted a protective chemotherapeutic effect in experimental infections of mice with *C. neoformans* and seems to be the first synthetic compound to show a definite effect in this infection.

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FRANKLIN C. BING | *Chicago, Illinois*

PROFESSOR LAFAYETTE B. MENDEL AS A MEDICAL ESSAYIST*

It was spring in New Haven in the year 1927. The elm trees were in leaf and all outdoors beckoned, as is fitting on sunny days in April throughout New England. In a classroom at 333 Cedar Street on this particular afternoon were Professor Lafayette B. Mendel and his students. The weekly seminar in physiological chemistry was in session. One of the women participants, who had just completed her contribution to the discussion, abruptly changed the subject.

"Professor Mendel," she said with animation, as though she were about to report a discovery, "have you seen last week's *Journal of the American Medical Association?* It has an excellent editorial on the subject of our seminar, and it says—"

At this point our excited fellow student paused to look through her notes, while some of the rest of us held our breath and watched the Professor intently. We were not sure, but we believed that he himself had written the editorial, and we wondered how he would respond to this direct inquiry of one of our less informed colleagues.

Without the slightest hesitancy, and without indicating in any way that he knew more about the matter than he was telling us, the Professor said he had seen the editorial and then proceeded with the development of the assignment for the day.

In all the time during which I knew him Professor Mendel never once mentioned that he was a regular contributor to the editorial pages of the *Journal of the American Medical Association,* yet we, his students, knew that only he could have written many of the essays. From time to time an editorial would appear which we would recognize as an epitome of one of our seminars, and so frequently did this occur that we scanned the *Journal* hopefully in an effort to learn beforehand how our assigned reading matter fitted into the general subject of the Wednesday afternoon sessions. This help was possible only when the seminar followed the publication of the editorial, which sometimes happened—to the delight of those of us who had been assigned a difficult paper, usually in German.

There was a great deal of secrecy connected with this activity of the Professor, probably because that was the wish of Dr. George H. Simmons,

* Read at a dinner meeting of Yale biochemists, Chicago, Illinois, April 7, 1953.

longtime Editor of the *Journal of the American Medical Association,* for reasons of policy which I am sure were desirable. I am informed that contributors of editorials identified themselves on manuscripts by a code number; in Doctor Mendel's case this was No. 46. Years later, in "A history of the American Medical Association, 1847 to 1947," Dr. Morris Fishbein wrote that during the time when he was assistant to Dr. George H. Simmons, from 1913 to 1924, "most of the editorials in the *Journal* were written by Drs. Lafayette B. Mendel, Ludvig Hektoen, and Morris Fishbein." Dr. Mendel's contributions to the editorial section continued regularly until the onset of his final illness in 1934.

The preparation of this paper took me naturally to the files of older volumes of the *Journal of the American Medical Association.* The issue dated March 19, 1927, of volume 88, is more or less typical of the *Journal* of those days. There were seven essays in the editorial section (pages 926 to 929), the titles of the major contributions being: "The President signs the Lye Bill," "The centenary of the birth of Lord Lister," and "Plastein in nutrition."

In addition, there were four shorter essays under the heading of Current Comment, with the following titles: "One hundred years of aluminum," "The Koch cure as a 'valve-handle wheeze,'" "Red meat and white meat," and "Diabetes and growth."

It would not be difficult to guess the author of at least one of these editorials. The long essay on plastein was undoubtedly written by Dr. Mendel; one of his seminars for graduate students at about that time, as I recall, was on the subject of synthetic proteins produced by the action of pepsin on digested proteins, the reverse of proteolysis. And the subject, the style, and the thought of the article are all typical of the Professor's writings.

Other essays in this issue likewise bear the "earmarks" of Mendelian articles. Of course, no one would know for certain who wrote them except the Editor, who was responsible for the content of each issue.

It is somewhat of a coincidence that two of the items in the issue of the *Journal* selected dealt with anniversaries of scientific achievements. The essay on Wöhler's isolation of aluminum, indeed, forecast another anniversary, then a year in the future. "Next year," the essayist wrote, meaning 1928, "will witness another centenary of a Wöhler achievement: the synthesis of urea, which disposed of the earlier supposition that the characteristic organic compounds found in animal tissues and fluids require some unique "vital" force for their production."

I well remember that the following year, in the issue dated June 30, there appeared an essay entitled "Friedrich Wöhler and urea, 1828," for this

essay was published on pages 2105 and 2106 of volume 90 precisely as I had written it, at Professor Mendel's request. Thus began a collaboration in editorial writing which continued for several years, while I was in Cleveland with Dr. Victor C. Myers. Sometimes I wrote articles on request; at other times the initiative was mine. This work, always gratuitously performed, constituted a remarkable course of training in the art of writing on scientific matters. The Professor's criticisms I carefully kept.

He made it clear that he merely submitted material for the consideration of the Editor, whose judgment was final. Thus, on one occasion, the Professor wrote ". . . why don't you send me an occasional brief story that might be suitable for editorial use in the *Journal of the American Medical Association?* You know the general style and the sort of topics that appeal; and I shall be glad to transmit what you prepare, if I think that it is likely to meet with approval."

A few excerpts from the Professor's correspondence with me during this period may be of interest. "Avoid *direct* quotations whenever possible," he wrote in criticism of one of my earlier efforts. "If you wish to refer to the results or opinions of others, attempt to incorporate them indirectly into your manuscript. I shall try to see whether the *Journal* of the A.M.A. will use the material, possibly after a little editing on my part."

When my contributions appeared in print in due course, I began a habit, which I found to be instructive, of comparing the published editorial with my manuscript and with the Professor's criticisms. At all times his critical comments were most graciously stated; always they were to the point. He was a perfectionist.

In time I earned such comments as: "The general plan and style of your reviews are good." Then he added, to show me, no doubt, that my training was by no means completed, "You will need to learn to cut down on the number of minor details such as figures and c.c., grams, etc., and other numerical details. The casual reader prefers to learn by comparative statements rather than through numerical statistics. I can see that you have studied the style of the editorial columns of the J.A.M.A."

There were, of course, several writing assignments of a different nature, but my purpose here is to deal with the editorial writings of the Professor. In doing so, I find it necessary to bring my own small part into the picture, for Dr. Mendel was reticent about his own affairs and it was only by accident that I learned of his early career in medical journalism, an area of postgraduate medical education on which I believe many would agree in placing high value. Dr. Mendel's editorials conveyed the enthusiasm of his seminars, so well described by Professor William C. Rose in the *Journal of*

Nutrition (1936, *11*, 607), to thousands of physicians nearly every week for many years. His editorials bore an authoritative message about newer developments in the medical sciences and pointed to the clinical significance of laboratory research. For years, the editorial section of the *Journal* was considered by many persons to be an outstanding feature of the publication, and the thoughtful essays contributed by Dr. Mendel certainly helped to establish and to maintain that reputation.

Once on a vacation trip to New Haven I chanced to pick up at Head's secondhand bookstore on Elm Street a lone volume of a journal which was then unknown to me. It bore the title *The Dietetic and Hygienic Gazette,* of which it constituted Volume X. Inside was the signature "Lafayette B. Mendel" followed by the date, which was 1895. I purchased the volume for the price asked, which was, as I recall it, ten cents, and subsequently mentioned it in a letter to Dr. Mendel. His reply came from Delhi, New York, under the date of July 29, 1933:

> Your finding of one of my volumes of the *Dietetic and Hygienic Gazette* [he wrote] was a surprise to me and brings back memories of the days of my academic youth. This journal, which was never a "heavy weight," and has long since been discontinued, had a department that was edited by Prof. Chittenden. At his request I often wrote reviews of scientific work then recently published. I continued this gratuitous activity while I lived abroad in 1895 and 1896. It offered me my earliest experience in abstracting and editorial writing. Years ago I removed the copies of the *Gazette* from my own library and deposited them in the old laboratory at 2 Hillhouse Ave. I presume that they were discarded into the "junk heap" of old books when we moved to Cedar St.; at least I cannot recall anything further about my copies. The first review that I wrote was a rather long one (probably in 1895) dealing with an elaborate investigation on inanition by Prof. Zuntz of Berlin.

The Professor's recollection of the date of his first editorial contribution to this publication proved to be in error. It was in 1893, which was the year in which he received his Ph.D. degree from Yale. These early contributions are readily identified, for they were published under his name. The titles of the Mendel reviews in volume X for 1894 will be of special interest to

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FIG. 1. The cover page of the journal in which Professor Mendel's first medical editorial was published; his academic degree is incorrectly stated on the cover. In the January, 1893, issue of this publication there appeared the following statement: "We cannot offer our readers a more pleasing announcement than that Prof. R. H. Chittenden, of Yale, has become a collaborator in the interest of readers of the *Gazette*. This gentleman has so long been identified with the Physiology of Nutrition that we regard his connection with this Journal as an important event. Prof. Chittenden will, as is indicated on p. 7, edit the department of Physiological Chemistry with special reference to dietetics and general nutrition."

The essay referred to, entitled "Normal diet," is a revealing statement of Professor Chittenden's views at that period of the development of nutrition. The first signed article by Dr. L. B. Mendel in this publication is a translation of a German article by Prof. A. Fick, "The importance of fat in nutrition," in the March issue, pages 58-60.

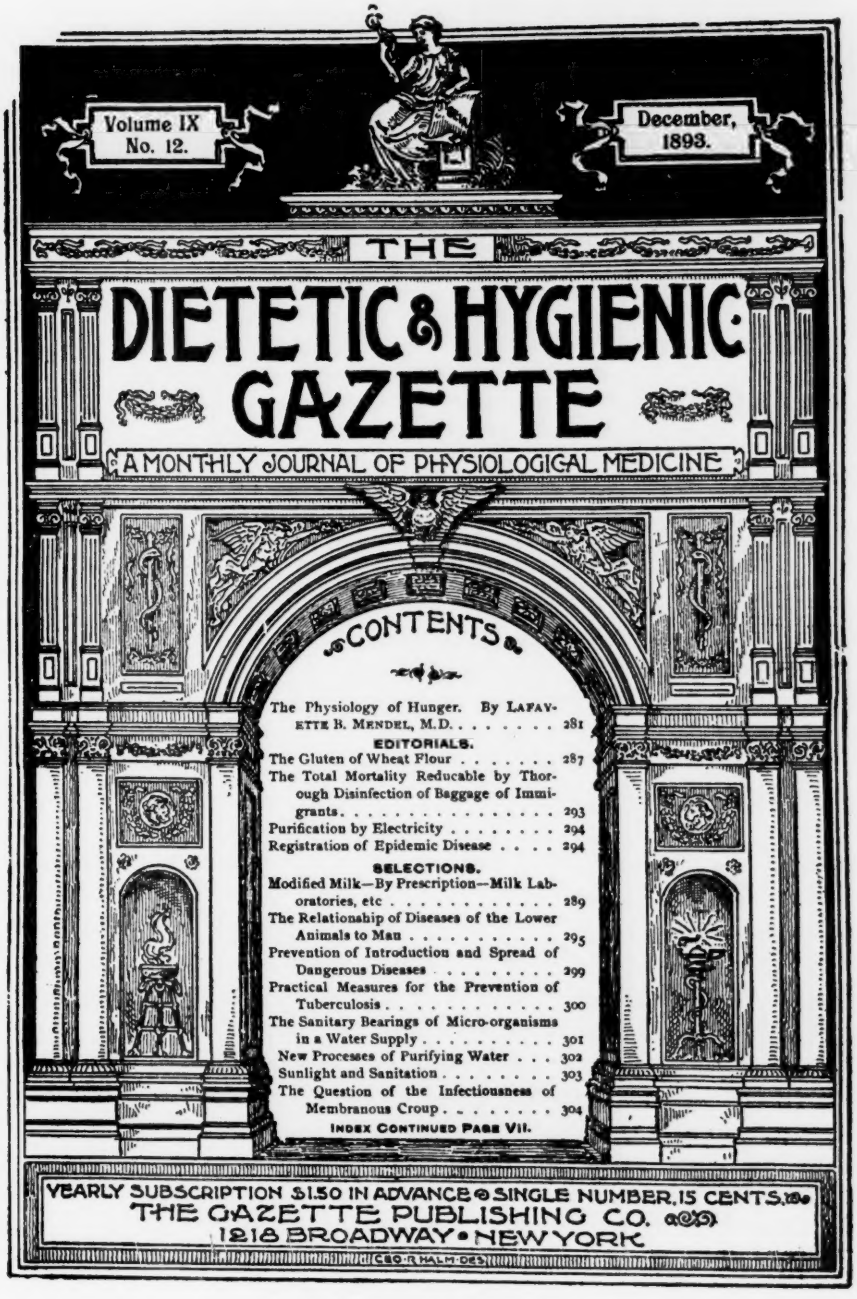
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FIG. 1.

some of his former students, who will recognize among them subjects which were of recurring interest to the Professor throughout his professional career. These titles were:

Digestion after gastrectomy, pages 119-121; Some observations on phlorhizin-diabetes, pages 121-122; Diet in tropical countries, pages 178-180; Some observations on the bread-cereals as footstuffs, pages 225-228; Food and animal heat, pages 328-330; Recent investigations on the relation of the nervous system to the glycogenic function of the liver and diabetes, pages 381-385.

Later on I learned that Professor Mendel frequently contributed to the *Journal of the American Medical Association* editorials on purely clinical subjects. He was particularly interested, it seems, in medical reports on the treatment of heart conditions, but his range of interests was wide. "Often," Dr. Morris Fishbein has informed me, "he used to write editorials by hand while traveling on trains." Others who worked in the editorial department at the A.M.A. headquarters have told me the same story. The number of his contributions was amazing; there were occasions when every editorial in an issue had been contributed by the Professor. The quality of all his essays was uniformly excellent, for he wrote with a facile pen. The essays were stimulating at the time they were published, and they make informative and interesting reading today.

Early in 1936 I went to the American Medical Association offices in Chicago to serve as Secretary of the Council on Foods and Nutrition, a position which I held until 1943. One of my first duties on joining the staff was to approve the galley proof of Professor Mendel's obituary, prepared by others, for publication in the Council section of the *Journal.* It was indeed a sad duty to perform. The obituary did not seem to do him justice, but I doubt if anyone could have written an adequate description of the many worthwhile activities of this truly great man. Not the least of his services to science and medicine were the thoughtful essays on the results of current investigations, which he wrote for so many years.

He was first and last a teacher, whom some of us today were fortunate enough to have known and loved, just as I am sure that he loved most of us. He derived pleasure from the accomplishments of others, which is requisite to successful writing about them. Thoroughly acquainted with the scientific contributions of earlier workers, he was ever mindful of the opportunities for further research. He would, I am sure, have been pleased with the development of his specialties at the University where he worked, and with the new horizons for Yale students today in biochemistry and nutrition. His lifelong association with the University is something in which all Yale men may take pride.

HARRY SIGEL

Division of Dermatology, Department of Medicine, Yale University School of Medicine

PRICK THRESHOLD STIMULATION WITH SQUARE-WAVE CURRENT: A NEW MEASURE OF SKIN SENSIBILITY

Threshold sensory stimulation of the skin has become the object of increasingly intensive investigation since the classic report by Hardy, Wolff and Goodell[5] on the pain threshold of thermal radiation. The literature on the subject is rapidly becoming voluminous with interesting ramifications appearing in many fields of medicine. More recently Cormia[3] reported on the itch threshold in experimental histamine pruritus and still more recently Benjamin and Ivy[1] have described the flare threshold associated with the pain of thermal radiation. The purpose of this communication is to report studies on the prick threshold of the skin and its measurement with square-wave stimulation.

In previous studies I have found* that threshold cutaneous sensory stimulation could be achieved with square-wave current, a type which is essentially interrupted, unidirectional, and produces repetitive impulses. The frequency, duration, and strength of the impulses are regulated by the square-wave stimulator to provide threshold stimulation. It was also found that the size of the active electrode must be adequate (about two inches square) to cover a sufficient number of sensory units in order to produce consistent responses; and in person-to-person comparisons the same body site should be stimulated, since sensory responses are not uniform over the body.

Bishop[2] has shown that the prick fibers of the skin are highly versatile, mediating sensations which vary in quality from tactile and pressure sensations to frank pain, depending upon the intensity of the electrical stimulation; also that prick fibers have a lower threshold to electrical stimulation than those of touch fibers. It is thus clear that minimal electrical stimulation of the skin is necessarily prick threshold stimulation and that the resulting responses are the weakest mediated by the prick fibers.

In attempting to measure and standardize the prick threshold of the skin the chief problems were:

---

* This work was aided by a grant from the G. H. Knight Memorial Fund, Yale University School of Medicine.

Received for publication April 26, 1953.

1. To determine the relationship of each of the three parameters, frequency, voltage, and impulse duration, to threshold responses
2. To choose a standard threshold response, and
3. To evolve an accurate method of measuring the prick threshold with the three parameters.

APPARATUS

In the present investigation a Model S-4 Grass Square-Wave Stimulator was employed (Fig. 1) to which was connected a calibrated oscilloscope. The apparatus itself was accurately calibrated to within ± 5% accuracy for each parameter and was a much more efficient machine than was used in the earlier studies. It differed also from the apparatus previously used in that impulse duration could be read directly from the dial setting and was not a function of the frequency setting. However, care had to be taken not to exceed the 50% duty cycle* in order to insure calibration accuracy. In this apparatus the frequency range was from 1 cycle per 10 seconds to 10,000 cycles per second (cps.); voltage range was from 0.15v to 150v peak; and impulse duration ranged from .01 milliseconds (msec.) to 1000 msec.

The active electrode consisted of a curved EKG arm electrode measuring 1½ by 2 inches and padded with saline-moistened gauze. The indifferent electrode consisted of a large piece of lead-tin material similarly padded.

TECHNIQUE

The subject is placed on an examining table, relaxed and supine. The active electrode is applied to the normal skin of the volar surface of the upper forearm, where hair is minimal, and kept in place with the wide perforated rubber band that is customarily used with the EKG electrode. Very little tension is applied to the rubber band so as not to crease or depress the skin. The frequency and impulse duration parameters may be set for any desired reading while voltage is adjusted below the estimated sensory threshold. Voltage is then rapidly increased (about one volt per sec.) until sensation is perceived by the subject. Next, the voltage is decreased until sensation disappears, and increased again—this time more slowly to ascertain the minimal voltage required to produce threshold sensation. The minimal values for frequency and duration may be determined in the same manner.

INTERRELATIONSHIP OF IMPULSE DURATION, FREQUENCY, VOLTAGE, AND THE THRESHOLD RESPONSE

This portion of the study was based on prick threshold measurements taken on approximately 100 subjects, mostly patients attending the Grace-New Haven Community skin clinic but including some medical students, nurses, physicians, and the author. The subjects were asked to report the

* The duty cycle is one complete cycle from make to make, the duration of which depends upon the frequency. Thus, for example, if frequency is set at 200 cps., then there is a 5 msec. period between pulses and the duration should not exceed 2.5 msec.

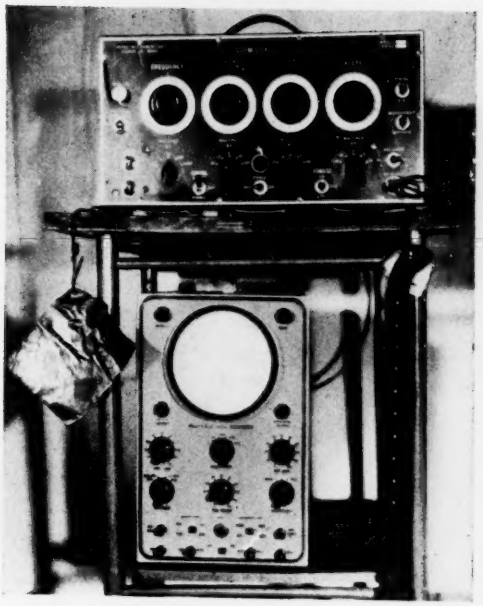

FIG. 1. Square-wave stimulator and oscilloscope.

slightest sensation or sensation they could "just barely feel" as soon as it was perceived, and on these reports, plus the author's own observations, was based the description of the responses. Although these measurements

TABLE 1. MINIMAL THRESHOLD VOLTAGE READINGS AND RESPONSE IN A WIDE RANGE OF FREQUENCIES AND IMPULSE DURATIONS IN AN INDIVIDUAL (AUTHOR).

At 10 msec. impulse duration, burning threshold response was produced at each frequency. At 0.1 msec. impulse duration, burning threshold response began at about 1000 cps. With 0.01 msec. impulse duration, burning response began at about 500 cps. Minimal threshold voltage was almost steady above 1 msec. impulse duration and began to climb below 1 msec. impulse duration, rising sharply at 0.01 msec. impulse duration. At frequencies below 10 cps., the response was tapping to pulsating; from 50 cps. to 500 cps., vibratory to prickly; above 500 cps., burning.

Frequency	*Duration*	*Response to shock*	*Volts*
1/10/sec.	10/ms.	Tapping, burning	9
1/sec.	"	Tapping, burning	9
10/sec.	"	Pulsating, burning	8.5
50/sec.	"	Vibrating, burning	8.5
1/10/sec.	1/ms.	Tapping	9
1/sec.	"	Pulsating	9
10/sec.	"	Pulsating	8.25
50/sec.	"	Vibrating, prickly	8
100/sec.	"	Vibrating, prickly	7
500/sec.	"	Vibrating, prickly	7.75
1/10/sec.	0.1/ms.	Tapping	21
10/sec.	"	Pulsating, light prick	21
50/sec.	"	Vibrating, light prick	20
500/sec.	"	Vibrating, light prick	20
1000/sec.	"	Vibrating, burning	10.5?
5000/sec.	"	Burning	25
1/10/sec.	0.01/ms.	Tapping	53
10/sec.	"	Pulsating	47
50/sec.	"	Vibrating, light prick	45
500/sec.	"	Burning	45
5000/sec.	"	Burning	57
10,000/sec.	"	Burning	47.5

were carried out under ordinary clinic conditions, no difficulty was encountered because of this circumstance. The patients were able to concentrate well, although they occasionally failed to report the initial sensation because they were waiting for more intense stimuli, or sometimes reported sensa-

tions when no stimulus was being delivered. With experience these errors were easily recognized and rectified.

Threshold responses. Three general types of threshold responses were elicited: (i) tapping, (ii) vibrating, and (iii) burning. Each of these was elicited within certain limits of frequency, duration, and voltage (Table 1).

Effect of frequency. With frequencies of 1 cycle per 10 secs. to 10 cps. (and impulse duration less than 10 msec.) responses were tapping to pulsating, tactile in character. These sensations correspond to the tactile and contact responses to single electrical stimuli of the prick fibers described by Bishop.[3] Frequencies above 50 cps. produced sensations described as vibrating, pressure sensation, fuzzy, tickling, or slightly pricking. These vibratory sensations merged into burning sensations above certain frequencies as influenced by impulse duration.

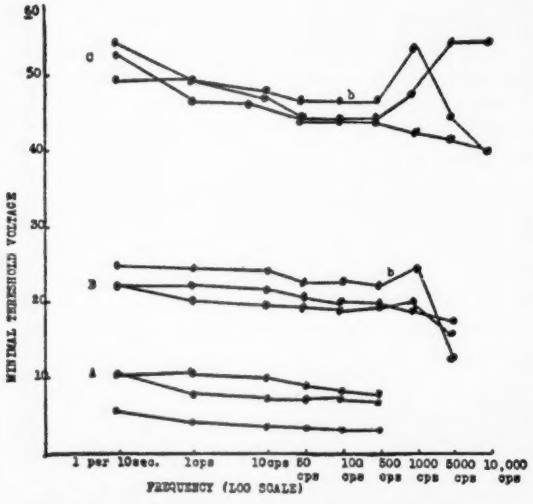

FIG. 2. Frequency plotted with voltage from three sets of readings with impulse duration at 1 msec., 0.1 msec., and 0.01 msec. This illustrates the slight influence of frequency and the great influence of impulse duration on minimal threshold voltage.

Effect of duration. With impulse duration at more than 10 msec., the response was invariably one of burning, even with the lowest frequency. At 1 msec. vibrating responses were perceived up to 500 cps. (the limit set by the 50% duty cycle). With 0.1 msec., vibratory responses were perceived up to about 700 cps., and with 0.01 msec., up to 300 cps., above which burning developed.

Effect of voltage. The quality of the threshold responses was not affected by the voltage, its effect being entirely quantitative. If the voltage was below threshold, there was no response. Above threshold, increasing voltage produced prick-pain with an after-discharge of burning. Still higher voltages produced greater discomfort and muscular contractions.

Minimal voltage was slightly higher with very low frequencies (producing tapping, pulsating responses) than with higher (vibratory) frequencies,

becoming erratic with burning responses. When voltage is plotted with frequency (and duration is constant), a horizontal curve is produced which is slightly raised at the start, almost flat in the center, and ends with erratic changes (Fig. 2).

However, minimal voltage was profoundly affected by impulse duration, rising rapidly as duration decreased. Voltage plotted with duration (frequency constant) produces a hyperbola (Fig. 3).

Choice of threshold response. Burning responses were eliminated in the choice of a standard type of response because of (i) the erratic character of the threshold, (ii) the after-discharge which prevented immediate restimulation, and (iii) the development of erythema at the site of the active electrode after short periods of stimulation. The vibratory response was selected in preference to the tapping-pulsating type of response because measurement of the latter was more time-consuming.

In order to further standardize the response it was necessary to employ the same frequency for all readings. The frequency of 50 cps. was selected because it permitted the use of impulse duration from 0.01 msec. to 10 msec. within the 50% duty cycle, higher frequencies not being as flexible.

METHODS OF MEASURING PRICK THRESHOLD

Rheobase-chronaxie and strength-duration methods. When minimal voltage is plotted with impulse duration (frequency being constant), a typical curve is obtained (Fig. 3). This curve is analogous to the curve of excitability commonly recognized by physiologists as derived from threshold stimulation of nerve or muscle tissue. Lapicque[6] based his original measurements of rheobase and chronaxie on this universal curve of excitability. By definition, rheobase is the lowest voltage which will produce stimulation of excitable tissue, below which no response is obtained even with infinite duration of the stimulus. Chronaxie is defined as the duration of stimulus necessary to produce a threshold response with twice the rheobase voltage.

The rheobasic voltage or galvanic threshold is readily obtained on this square-wave stimulator by tripping the DC switch which automatically shuts off square-wave current while turning on galvanic current. The voltage is set below the estimated threshold and then gradually increased until the subject perceives the initial sensation. This is usually reported as a burning, pricking, itching, or tapping sensation. The minimal voltage reading is the rheobasic voltage. Chronaxie is obtained by setting voltage at twice that of rheobase and selecting the minimal impulse duration to obtain

the vibratory threshold response (frequency at 50 cps.) with square-wave current.

The other obvious way of measuring prick threshold levels is to select a constant frequency and duration and to measure the minimal voltage producing threshold sensation. With 50 cps. frequency, minimal voltage could thus be measured at 10 msec., 1 msec., 0.1 msec., or 0.01 msec. duration (strength-duration method).

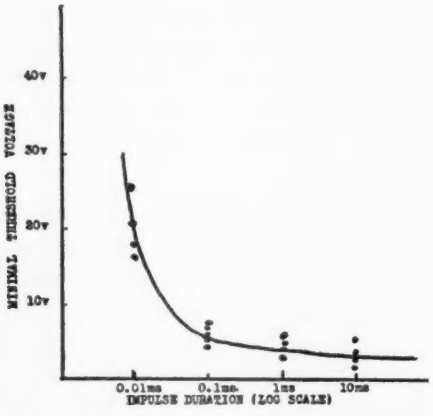

FIG. 3. Minimal threshold voltage plotted with impulse duration (frequency at 50 cps.) from five sets of readings. With impulse duration below 1 msec., voltage begins to rise. This rise becomes very sharp at impulse duration below 0.1 msec.

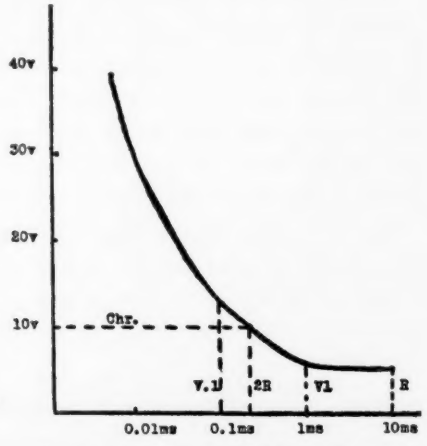

FIG. 4. Graphic illustration of the relationship of Rheobase (R), Chronaxie (Chr.), and minimal threshold voltage at 0.01 msec. (V. 1). The curve of excitability becomes critical with impulse durations below 1 msec. Both 2R and V. 1 readings fall on the critical portion of the curve.

Rheobase-chronaxie. The use of two separate readings, rheobase and chronaxie, was felt to be less desirable than a single figure to represent prick threshold measurement. Lassalle[7] has proposed the reciprocal of rheobase[2] x chronaxie as an "index of excitability" of tissues. It was soon found feasible to employ this formula and to express the prick threshold in terms of the index of excitability. One hundred ninety-seven readings on 177 subjects were taken over a period of six months with the rheobase-chronaxie method described above, and the index of excitability determined with Lassalle's formula. As a result of these readings it was found that the galvanic threshold was not always satisfactory for determining rheobasic voltage. With a threshold response of burning, the minimal voltage determination was often erratic, and because the stimuli were necessarily single, the voltage was frequently higher than with repetitive stimuli at 10 msec.

or even 1 msec. impulse duration. Usually minimal voltage differs only slightly between galvanic rheobase and voltage at 10 msec. duration and any significant differences were felt to be due to the erratic nature of the galvanic rheobase. For these reasons the minimal voltage at 10 msec. impulse duration was adopted as the rheobase value for prick threshold measurements. To date more than 150 readings on almost as many subjects were

TABLE 2. PRICK THRESHOLD MEASUREMENTS IN TEN NORMAL INDIVIDUALS ILLUSTRATING THE METHOD OF DERIVING THE INDEX OF EXCITABILITY

Rheo. – rheobase, minimal voltage at 10 msec. duration; V1 – minimal voltage at 1 msec. duratoin; V. 1 – minimal voltage at 0.1 msec. duration; Chr. – chronaxie, minimal duration at twice rheobasic voltage; I.E. – index of excitability derived from the formula $\frac{1}{Rh^2 \times Ch}$.

Case no.	*Rheo.*	*V1*	*V. 1*	*Chr.*	*I.E.*
1	6.5	6.5	10.5	0.05	0.4
2	3.5	3.5	9	0.15	0.5
3	4.5	6.5	13	0.15	0.3
4	4	8	14	0.2	0.3
5	6	6	11	0.085	0.3
6	10	10	12	0.03	0.3
7	3.5	6	9	0.2	0.4
8	4	7.5	14.5	0.3	0.2
9	8	8	11.5	0.05	0.3
10	5	5	7	0.055	0.7

taken with this method with greater accuracy and reliability than was formerly achieved.

Strength-duration. At the same time that the above readings (with both types of rheobase) were taken, threshold voltage measurements on many of the same subjects were also made at 1 msec. and 0.1 msec. durations. Correlations were sought between strength-duration, rheobase, chronaxie, and the index of excitability. The only correlation which was found was between the voltage at 0.1 msec. and the index of excitability. This could be expressed by taking the reciprocal of half the minimal voltage at 0.1 msec. (V. 1) squared times 0.1:

$$\frac{1}{\left(\frac{V.\ 1}{2}\right)^2} \times 0.1 \text{ equivalent to } \frac{1}{Rh^2 \times Ch} = \text{I.E.}$$

The values expressed by both formulae coincided very closely.

The explanation for this appears on examination of the curve of excitability (Fig. 4). The minimal voltage at 1 msec. duration falls on the flat portion of the curve and is often the same as at 10 msec. duration. The minimal voltage at 0.1 msec. falls on the arched portion of the curve where changes in voltage are commensurate with changes in duration. The twice rheobase voltage also falls on this portion of the curve where, conversely, duration varies commensurately with voltage. Frequently, the twice rheobasic voltage was found to be the same as voltage at 0.1 msec. with chronaxie also coinciding at 0.1 msec. Both sets of measurements thus serve to double check each other.

In order to determine the index of excitability for each subject, readings are taken at the following settings of the apparatus:

1. Frequency at 50 cps.
2. Minimal voltage determined at 10 msec. impulse duration (rheobase)
3. Minimal voltage determined at 1 msec. impulse duration
4. Minimal voltage determined at 0.1 msec. impulse duration
5. Minimal duration determined at twice rheobasic voltage (chronaxie)

(With these readings one may actually plot a curve of excitability for each individual.) The index of excitability is computed by the formula $\frac{1}{Rh^2 \times Ch}$ and is checked by the formula $\frac{1}{\left(\frac{V.\ 1}{2}\right)^2} \times 0.1$. Table 2 shows the readings taken on 10 normal subjects to illustrate this method.

THE INDEX OF EXCITABILITY IN NORMAL PERSONS

The index of excitability of the skin was determined in 55 normal persons with the above method. The subjects consisted of medical students, nurses, and physicians with normal skin who were apparently normal physically. The index in this group ranged from 0.1 to 0.8 with 78% falling between 0.1 and 0.4 (Fig. 5). The average and the mean were both 0.3, with a standard deviation of 0.18 and standard error of .023.

DISCUSSION

Although much has been written on muscle and nerve chronaxie, I have been unable to find any reference in the literature concerning cutaneous sensory chronaximetry. Davis and Forbes[4] in a comprehensive review of chronaxie emphasize the arbitrary and empirical nature of this measurement and stress the importance of a standardized rheobase. For these

reasons it was felt that the shift of rheobase in these studies from the galvanic threshold to the prick threshold at 10 msec. impulse duration was justified, inasmuch as the readings were more dependable at the latter setting.

The measure of cutaneous sensibility with square-wave stimulation evolved in this study may have wide clinical usefulness. Richter[8] has described the relationship of the electrical resistance of the skin to psychic, emotional, and organic disturbances. Although it was not found necessary to measure electrical resistance per se in determining the index of excitability, it is obviously an important contributory factor in the electrical measurements. Other avenues of usefulness for prick threshold measurements are indicated in the work of Hardy and his associates on pain thresholds, for prick thresholds and pain thresholds may be expected frequently to exhibit similar patterns of measurement. Furthermore, Benjamin and Ivy,[1] who showed relationship between the flare threshold and neoplastic and organic diseases, cite the possibility of cutaneous sensory axon stimulation to account for the underlying mechanism of their observations. This may prove a fruitful field for prick threshold measurements. Whether this new electrical measure of skin sensibility will provide a more accurate or more versatile biometric standard than previous methods will depend on the results of further studies. This much may be said for it at present, that it is a simple method, inexpensive, and of high reliability.

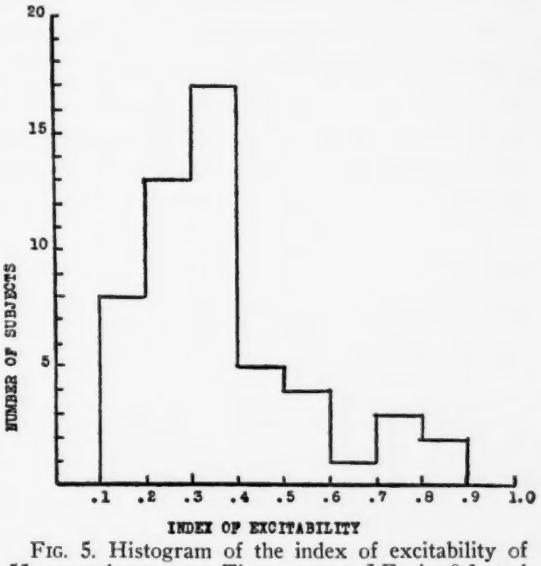

FIG. 5. Histogram of the index of excitability of 55 normal persons. The average I.E. is 0.3 and ranges from 0.1 to 0.8.

SUMMARY AND CONCLUSIONS

1. Prick threshold stimulation with square-wave current was studied by investigating the interrelationship of frequency, voltage, impulse duration, and the response.

2. A method of measuring prick threshold was evolved in which a constant frequency was employed (50 cps.) to produce a "vibratory" response at various levels of minimal voltage and impulse duration.

3. Plotting the relationship of minimal voltage to impulse duration produced a curve of excitability of cutaneous stimulation. This led to the employment of rheobase and chronaxie measurements and to the adoption of the reciprocal of rheobase^2 x chronaxie as an index of excitability.

4. A similar index was found by taking the reciprocal of the square of half the voltage at 0.1 msec. duration x 0.1. Both indices concide closely and serve to check each other.

5. In 55 normal persons the index ranged from 0.1 to 0.8 with 78% of the readings between 0.1 and 0.4. The average and the mean were both at 0.3.

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PROSPECTUS FOR A SURVEY OF HUMAN CANCER BASED UPON THE CONCEPT OF PARENTAL AGE

Cancer incidence is associated with the "ageing process." It is frequently stated that cancer is a disease of old age. This idea is based primarily upon the fact that both skin carcinoma and carcinoma of the breast increase in frequency with advancing age. In this case the relative frequency remains about the same. It has been maintained that the great increase in cancer mortality at the present time is due in part to the increased average expectancy of life in man.

The ageing of the individual may contribute toward the ageing of species to which the individual belongs. However, this theory is not supported by experimental evidence but it should not be dogmatically dismissed. It is not too much to keep in mind, therefore, that the ancestral descent, including parents and grandparents, may possibly contribute to cancer susceptibility in the individual.

The increased expectancy of life or longevity has been due to several causes among which may be mentioned the improvement in the treatment of diseases of infancy, advancements in medical care, and the improvement of public health by sanitation, immunization, and other practices. The recent introduction of antibiotics has already influenced the average expectancy of life. It is probably true that the life expectancy at sixty is about the same now as it was fifty years ago. The nature of man has not changed, although Pearson has maintained that there has been a selective survival-time influence during the past three centuries, the greater proportion of the population belonging to large families derived from parents who lived beyond the average expectancy of life.

The problem of longevity is a complex one. The forces at play are intrinsic or genetic and environmental. Again cancer susceptibility, in both experimental animals and in man, is a complex variable. It is probably unwise to draw final correlations between two complex systems. It becomes necessary, therefore, to resolve the problems of the expectancy of life and of cancer susceptibility into their component parts.

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Four investigators have shown in experimental animals that maternal age influences cancer susceptibility in the offspring. These are Bittner, working with adenocarcinoma of the mammary gland, MacDowell and Law investigating leukemia, and Strong with chemically induced fibrosarcoma. These studies have all been done on mice and all three tumors are apparently biologically different.[1]

Longevity in man is influenced by (i) maternal age at the time the child was born (Jalavisto), (ii) the number of children in the family (Beeton, Yule, and Pearson), (iii) the decrease of infant mortality with advancing age of parents (Ploetz), (iv) the age at death of the parents (Jalavisto and Ploetz), and probably by many other factors which are yet unknown. A complete bibliography and a summary showing the present status of the problem of parental age is being published as a monograph on *Parental age and characteristics of the offspring* by the New York Academy of Sciences.

The experimental work on chemically induced fibrosarcomas in mice[1-8] has shown that the ordinal sequence of litters influences fibrosarcoma susceptibility, obviously a reflection of maternal, if not parental, age,* and that the size of the family determines trends in cancer susceptibility.

A summary of research, upon which the first statement above is based, is as follows: The present study deals with the appearance of fibrosarcomas in mice following the subcutaneous injection of methylcholanthrene at sixty days of life. For this purpose, 1,806 mice have been used, of which 1,115 were females and 691 males. The mice belonged to one original inbred strain of 30 generations which was continued by brother to sister matings exclusively. Three sublines have been continued: the original inbred, and two selected descents from parents belonging to first or second litters (early litter descent) or the 7, 8, or 9th litters (advanced litter descent). Divergence in the appearance of fibrosarcomas has been obtained in the two selected descents which amount to 28.0 $\pm$ 2.64 days. This difference is 10.6 $\times$ P.E. The evidence thus demonstrates that the principle as measured by litter seriation (ordinal sequence of the family) which affects chemically induced fibrosarcomas in the offspring is cumulative. A selection toward the suppression of the size of the family increased susceptibility (as measured by latent period or time between the injection of methylcholanthrene and the appearance of tumors) to chemically induced fibrosarcomas.

The second conclusion above is based upon the following observations. A total of 1,309 mice of four inbred descents, Prunt, 2Prunt, Pridil, and

* Haushka has shown (to be published in *Parental age and characteristics of the offspring*) that litter seriation and maternal age have the same influence on "open eyelid" in mice.

IpBr have been injected with 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age; they developed fibrosarcomas at the site of injection and died. The mice belonged to the 1 to 12 litters. The characteristics of (i) latent period for the appearance of tumors, (ii) survival time with growth of tumors, (iii) age at death, (iv) a sex differential, and (v) invasiveness or ability to invade into the abdomen are analyzed in terms of litter seriation. A brief review of the literature is given and the problem of tissue specificity is cited.

Mice in successive litters show differences in susceptibility to chemically induced fibrosarcomas (latent period) and in characteristics of malignancy (survival time and invasiveness). There seems to be an inverse relation between survival time and latent period, although two mechanisms appear to be evident. In the Prunt strain female mice develop tumors earlier in succeeding litters, even though the males do not do so, thus bringing about a sex differential in relation to fibrosarcomas which increases in successive litters. Female mice show more tumors with the ability to invade into the abdomen than do males. The number of mice with malignant tumors (invasiveness) is reduced in mice of both sexes in successive litters. Survival time is also influenced by litter seriation.

The present data indicate two hereditary mechanisms involved in cancer susceptibility and malignancy which change during the life span of a female mouse and are handed down to her progeny. This transmission is apparently not by way of the chromosomes or genes but may involve cytoplasmic inheritance.

Literature on the size of family in man (except to show that it is getting progressively smaller in the United States), on either longevity or cancer susceptibility is not available. The age of the parents at the time of the birth of the offspring has not been adequately investigated in man either as a possible cause of increased expectancy of life or of alterations in cancer susceptibility.

It is proposed now by a group of investigators representing the various biological fields, such as genetics, gerontology, medicine, public health, etc., to pursue the following program on human subjects.

1. Ascertain data on parental (particularly maternal) age at birth of cancer proband, and to compare these data with a similar group of controls (non-cancer) of approximately the same age and circumstances.

2. Determine the ordinal sequence of the family for cancer patients and their controls.

3. Ascertain data on vital statistics for older and younger brothers and sisters to cancer patients and their controls.

Since the size of the American family is becoming smaller (4.9 in 1890 to 3.56 in 1950), the present survey should be of a preliminary nature because most people at present belong to small families. The final evaluation should be placed on the complete analysis of the large family.

This preliminary program should indicate (i) where cancer has occurred in a large family, (ii) the most likely types of human cancer that should be more carefully investigated, (iii) the causes of death of brothers and sisters of cancer patients and their controls, and (iv) whether the differences in cancer susceptibility between children of subsequent births are great enough to warrant a more extended investigation over a period of years of the possible cumulative effect of maternal age or restriction of the size of the family on cancer in the human offspring.

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THE EFFECT OF WHOLE BODY X-IRRADIATION ON THE RETICULO-ENDOTHELIAL SYSTEM AS DEMONSTRATED BY THE USE OF RADIOACTIVE CHROMIUM PHOSPHATE**

The rôle of the reticulo-endothelial system (RES) in recovery following whole body X-irradiation has aroused considerable recent interest.[4, 5, 17] However, ready explanation of this rôle is complicated by the fact that pertinent data concerning the effect of ionizing radiation upon the RES seem somewhat contradictory.[6, 8, 21, 23, 24, 29, 34]

Generally, the RES is regarded as a non-radiosensitive tissue.[3, 25, 32] Taplin, *et al.*,[28] reported very little effect of 300 r whole body X-irradiation on the RES of rabbits, basing their conclusions on the blood clearance of the colloidal dye, prodigiosin. Barrow and co-workers[1] were unable to demonstrate any change in the disappearance rate of intravenously injected colloidal gold into rabbits following 800 r whole body X-irradiation. On the other hand, Koga[20] noted an alteration in uptake of carmine by the RES of rabbits after only 42 r whole body X-irradiation, and, after 25 to 50 r, Pape and Jellinek[22] reported degenerative cell injury in the spleen of rats.

A technique has been developed by one of us[9–13] for study of RES activity in laboratory animals by means of intravenous injection of radioactive colloidal chromium phosphate ($CrP^{32}O_4$). The technique may be used in at least three ways:

(i) Disappearance rate of radioactivity from the blood may be determined. Thus, one can obtain an over-all outlook of the activity of the whole RES.

(ii) Distribution of radioactivity to different organs may be measured. Thus, the relative RES activity of various organs can be observed because, in normal animals, the distribution patterns of a $CrP^{32}O_4$ suspension seems very constant.[10] The liver, spleen, and many other organs remove a definite

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portion of the colloidal matter, and any distortion in the distribution may be interpreted as an altered function of the RES.

(iii) Radioautography of different tissues may be performed. In the liver and spleen a characteristic distribution pattern can be noticed,[10] but alterations of RES activity appear to change the usually constant pattern.

In this study, following small and moderate doses of X-irradiation, RES activity was assessed in rats using the radioactive colloidal chromium phosphate technique.

METHOD

Young adult, Wistar strain, male rats were used in all of the experiments. Body weights ranged from 120 to 150 g. Whole body X-irradiation was carried out with the use of a 200 KV X-ray machine at 15 mA. Filters of 0.5 mm. Cu and 1 mm. AL (HVL 1.1 mm. Cu) were used at a 50 cm. distance with an output rate of 55 r per minute in air.* Groups of five rats were irradiated at a time by placing them in small, individual cardboard boxes which were arranged to receive equal radiation.

Radioactive chromium phosphate suspensions were prepared as previously reported.[9] Briefly, anhydrous chromium phosphate containing P^{32} was dispersed in physiological glucose by means of a mechanical shaker. Suspensions used in this work were prepared as uniformly as possible in an attempt to keep the range of particle size relatively constant. Electronmicroscopic study of one preparation revealed an average particle size of 0.1 μ with a range from 0.01 μ up to several micra.†

EXPERIMENT A

Effect of X-irradiation on the removal rate of $CrP^{32}O_4$. Forty-eight rats were arranged in three groups of 16 each. One group received 25 r whole body X-irradiation, the second group received 50 r, and the third group, 100 r. Twelve additional rats served as controls. At different intervals from 1 to 28 days after irradiation, each rat was anesthetized with ether and the saphenous vein exposed in the thigh. Into this vein was injected 0.6 ml. $CrP^{32}O_4$ suspension. Immediately thereafter, the tip of the tail was cut off and seven blood samples of 0.05 ml. each were taken within 2-4 minutes. Radioactivity of the samples was determined as previously described,[9] and the radioactivity values were plotted against time elapsed after the injection. As reported before,[9] the disappearance rate of the radioactive colloidal matter from the blood stream occurs as an exponential function. Since this function seems to be very constant, the disappearance rate can

* For dosimetry calculations and X-irradiation, the authors wish to thank Dr. C. H. Chang, Department of Radiology, Yale University School of Medicine.

† Electronmicroscope pictures were obtained through the courtesy of Dr. J. L. Melnick and Dr. W. H. Gaylord, Department of Preventive Medicine, Yale University School of Medicine.

be expressed in terms of a "biological half-life," defined as the time required for one-half of the radioactivity to disappear from the blood stream.

No deviation of the biological half-life, when compared to control values, occurred in the experimental animals during 28 days following irradiation. That is, X-irradiated rats gave biological half-life values which were within the standard deviation of the control values.*

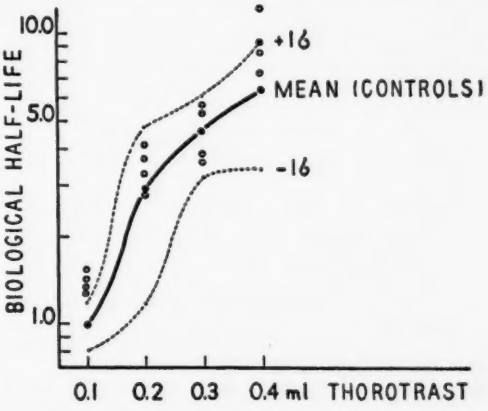

FIG. 1. Disappearance rate of colloidal $CrP^{32}O_4$ in rats 24 hours after intravenous injection of varying amounts of thorotrast.

Ordinate: Biological half-life (time required for one half of radioactivity to disappear from blood stream) in minutes (logarithmic scale).

Abscissa: Dose of Thorotrast in ml. per 100 g. body weight.

·—· Mean of 8 control animals; (· - - - - - ·) standard deviation.

o Values for rats irradiated with 100 r one week before.

EXPERIMENT B

Effect of X-irradiation on the capacity of the reticulo-endothelial system. Capacity of the RES was determined by intravenously injecting increasing amounts of colloidal thorium oxide (thorotrast†) into rats and subsequently testing the RES activity by means of $CrP^{32}O_4$. Sixteen rats were irradiated with 100 r and six days later divided into four equal groups. Thirty-two rats were added as controls, and (a) 12 rats (4 irradiated and 8 non-irradiated) were injected intravenously with 0.1 ml. Thorotrast per 100 g. body weight; (b) 12 rats received 0.2 ml.; (c) 12 rats 0.3 ml.; and (d) 12 rats 0.4 ml. Twenty-four hours later each of the 48 animals was injected with 0.7 ml. $CrP^{32}O_4$ suspension (see Experiment A), blood samples were taken, and the disappearance rate of radioactivity from the blood was determined.

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* The authors are appreciative of the assistance of Dr. Daniel Horn, Department of Public Health (Biostatistics), Yale University School of Medicine, in the statistical evaluation of these data. There are many statistical problems involved in the $CrP^{32}O_4$ technique. Quite a few of these have been discussed in previous publications.[9, 10, 11, 12] However, a general review article covering methodological problems and statistical evaluation is now in preparation.[14]

† The authors are grateful to the Heyden Chemical Corporation, New York, New York, for the samples of this preparation.

The results of this experiment are shown in Figure 1. In group (a), rats injected with 0.1 ml. thorotrast, the longer biological half-life values for the X-irradiated animals were statistically significant from the control values ($P < 0.01$). In fact, all X-irradiated rats of this group gave biological half-life values which were outside the standard deviation of the control values. In groups (b), (c), and (d), rats receiving greater doses of thorotrast, no prolonged half-life values for X-irradiated animals could be demonstrated when compared to control values. However, in these groups the spreading of the values was considerable, due to a greatly decreased RES capacity caused by the large amount of injected thorotrast.

TABLE 1. (EXPERIMENT C.) THE NUMBER OF RATS TESTED AT DIFFERENT TIMES AFTER 25, 50, AND 100 R X-IRRADIATION, AND THE CORRESPONDING GROUPS OF CONTROL ANIMALS

(Each group of 7 or 8 controls represents a different $CrP^{32}O_4$ preparation)

	Time from irradiation to $CrP^{32}O_4$ injection									
Radiation dosage	*1 hour*	*16 hours*	*24 hours*	*3 days*	*7 days*	*10 days*	*14 days*	*21 days*	*28 days*	*Total*
25 r	3	3	4	3	3	3	3	3	3	28
100 r	3	3	2	3	3	3	3*	3	2	25
50 r	3	3	4	3	3	3	3	3	3	28
0 r (Control)	7			8		7	8		**	30

* An additional 7 irradiated and 7 control animals were tested in this group.

** The 28-day group was tested with the same $CrP^{32}O_4$ suspension as the 10- and 14-day group and the 10- and 14-day controls served as a comparison.

EXPERIMENT C

Effect of X-irradiation on the distribution of $CrP^{32}O_4$ in different organs. Eighty-four rats were divided into three groups of 28 each and irradiated with 25, 50, and 100 r respectively. At intervals from one hour up to 28 days after irradiation, 0.7 ml. of $CrP^{32}O_4$ suspension was injected intravenously, and two hours later the animals were sacrificed. Using a technique described in detail elsewhere,[10] tissue samples were taken in sequence from tissue displaying the lowest radioactivity to that having the highest activity. The order was one sample each from abdominal muscle, duodenum, heart, and kidney, two each from spleen and lungs, and five from liver. All samples were weighed immediately; in the instance of the liver, the total organ was weighed. Samples were then ashed in Coors capsule-form cruci-

bles and the radioactivity of each was measured in a constant position under a bell-type Geiger-Mueller tube.

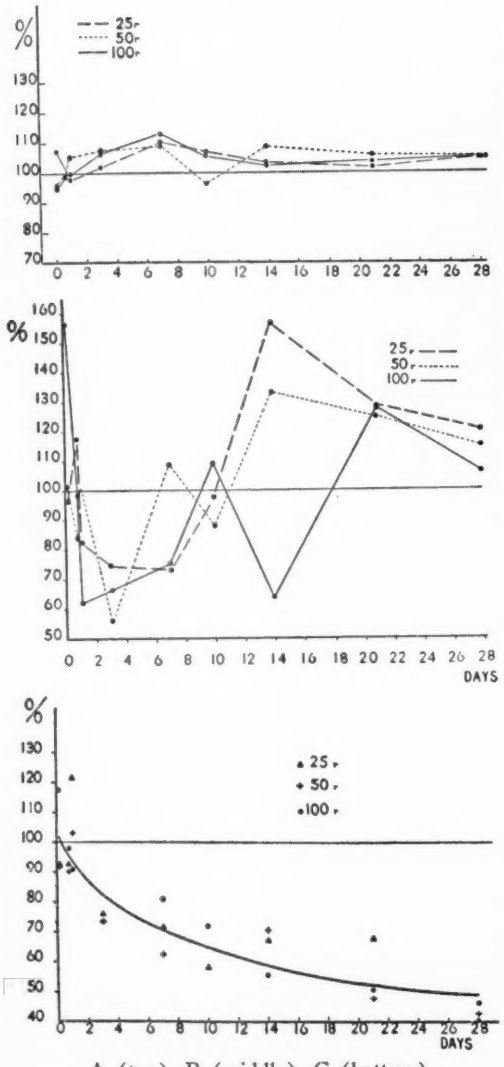
The three groups of X-irradiated animals were sampled 3 or 4 at a time at various intervals. Since the number of animals that can be processed at one time is limited, such an extensive experiment needs to be carried out in several sessions. A different $CrP^{32}O_4$ suspension is required for each session, thus, the measurements of radioactivity vary according to the suspension used. In this experiment, four different batches of $CrP^{32}O_4$ suspension were used, one for each session. Therefore, it was necessary to run a control series of 7 or 8 animals for each suspension. The original plan called for 3 animals from each group (25, 50, 100 r), to be injected with $CrP^{32}O_4$ at the following intervals: 1, 16, and 24 hours, and 3, 7, 10, 14, 21, and 28 days. Table 1 shows how the experiment was carried out with 81 irradiated animals and 30 controls.

The results of this experiment are indicated in Figures 2A, 2B, and 2C. Various tissue radioactivity (counts per minute) of the irradiated animals is expressed as a percentage of the average radioactivity of the same tissue from controls receiving the same $CrP^{32}O_4$ suspension.

The liver of the irradiated rats (Fig. 2A) contained more radioactivity than that of the corresponding control animals throughout the entire experimental period, with the largest increase being observed at 7 days after irradition in all three groups of 25, 50, and 100 r. The difference between the experimental groups and the controls was highly significant statistically ($P < 0.001$). However, no statistical differences could be demonstrated among any of the three groups receiving different doses of irradiation.

Results obtained from the spleen are shown in Figure 2B and, compared to the liver, changes in the spleen are quite different. One hour after 100 r irradiation the spleen of $CrP^{32}O_4$ injected animals contained 56% more radioactivity than the controls. But at this time values for the 25 r and 50 r groups were within the range of experimental error (96% and 102%). At one and three days after irradiation, however, all these experimental groups showed a highly significant decreased radioactivity of the spleen ($P<0.01$). At the 10-day interval there was little difference between the X-irradiated animals and the controls.

On the 14th day, the spleen of the 25 r and 50 r animals contained much more radioactivity than the controls, but the 100 r group showed considerably less radioactivity. Because this was the only instance in which the radioactivity values of 25 and 50 r groups increased while radioactivity values of 100 r groups decreased, that is, the respective values diverged when compared to controls, an additional 7 animals were subjected to



A (top), B (middle), C (bottom)

FIG. 2. Changes of radioactivity content of (A) whole liver, (B) whole spleen, and (C) abdominal muscle (per gram of tissue) of rats after 25, 50, and 100 r whole body X-irradiation. The animals were injected with colloidal $CrP^{32}O_4$ at different times after the irradiation.

Ordinate: Percentage values from irradiated animals taking control values as 100%.

Abscissa: Time after irradiation in days. Each point represents mean of three irradiated animals, sacrificed 2 hours after $CrP^{32}O_4$ injection.

C: Curve is calculated best fitting line.

100 r, raising the total tested in this group to 10. The additional 7 animals also showed decreasing radioactivity values when compared to those of an additional 7 controls. Therefore, this diverging difference between the 25 and 50 r groups as compared to the 100 r group was tested statistically and proved to be highly significant ($P < 0.001$). At the 21- and 28-day intervals, the differences converged, and all irradiated spleens displayed somewhat similar radioactivity values which, however, were still greater than the respective ones for the controls.

Heart, kidney, and lung samples showed no undue deviations of radioactivity values from those of corresponding controls. Two exceptions may be noted: at the 21-day interval all experimental values were lower than the controls; during the entire experiment abdominal muscle samples showed a continuous decrease of radioactivity (Fig. 2C).

DISCUSSION

When some damage to the RES is induced, the degree of reduced activity observed seems to be determined by two factors of opposite effect.

On one hand, the damage should depress the over-all RES activity, while, on the other hand, the "regeneration" of the RES should tend to re-establish the usual activity. Following an appropriate intravenous dose of a colloidal matter such as thorotrast or certain iron colloids,[11, 13] a large portion of the RES becomes saturated and "regeneration" seems to occur slowly. For several days after this saturation or "blockade" the RES displays a depressed activity which can be demonstrated by determining the biological half-life with the $CrP^{32}O_4$ technique.

After inducing colloidal iron "blockade" the $CrP^{32}O_4$ leaves the circulation very slowly.[11] However, injection of the dye, trypan blue, results in no noticeable "blockade" when assessed by use of $CrP^{32}O_4$, rather the radioactive colloid leaves the circulation somewhat faster than in noninjected animals.[13] Nevertheless, the histological picture indicates that many RE cells appear to be completely filled up with trypan blue dye. Apparently, a rapid "regeneration" of the RES overbalances the "blockade" in this instance and results in a slightly decreased biological half-life in the injected animals as compared to the controls.

In rats exposed to X-irradiation, no difference in the removal rate of $CrP^{32}O_4$ was noted from that of control rats (Experiment A). This observation is in good agreement with the work of Taplin *et al.*,[28] and Barrow and co-workers.[1] However, one should not assume that X-irradiation does not affect the RES, because X-irradiation damage could conceivably be balanced by an RES "regeneration," resulting in a $CrP^{32}O_4$ disappearance rate indistinguishable in irradiated and nonirradiated animals. As was pointed out in the previous paragraph, such an interpretation would find support from experimental observations using trypan blue.[13]

In order to demonstrate the above hypothesis, varying degrees of "blockade" were induced in 100 r X-irradiated animals and in controls by injecting varying amounts of thorotrast and subsequently testing RES activity by means of $CrP^{32}O_4$ (Experiment B). When as little as 0.1 ml. thorotrast per 100 g. body weight was injected into X-irradiated animals, the $CrP^{32}O_4$ did not leave the circulation as fast as in controls. That is, the X-irradiation plus the 0.1 ml. thorotrast "blockade" seemed to produce an additive effect, and "regeneration" of the X-ray damaged RES did not appear equivalent to the nonirradiated intact RES. When larger doses of thorotrast were injected, the degree of "blockade" was so large in the control animals that the minute difference between the X-irradiated and control animals was no longer demonstrable. However, the experiments with the 0.1 ml. dose of thorotrast seem to suggest that the capacity of the RES of a rat exposed to

100 r X-irradiation is reduced when compared to that of nonirradiated animals.

In the distribution studies (Experiment C), evidence indicates that all of the three doses of X-irradiation used in these experiments alter RES activity. In normal animals, the liver and spleen remove constant amounts of the intravenously injected colloid, but X-irradiation distorts the characteristic distribution pattern. This observation may be explained by a consideration of the particle size of the $CrP^{32}O_4$. The liver may constantly remove a definite range of particle sizes and so may the spleen or bone marrow. Following X-irradiation this selectivity may be distorted, and particles usually picked up by the spleen during the first week after X-irradiation would be taken up by the liver or other tissues. In this experiment, the selectivity of the RES components in removing the $CrP^{32}O_4$ particles from the blood stream appears to have been changed after only 25 r whole body X-irradiation.

Radioautographic studies add further support to the above observations, suggesting that the RES is radiosensitive. By means of radioautography, a characteristic distribution pattern of $CrP^{32}O_4$ in the liver was noticed.[16] This circular pattern may be due to the direction of blood flow from periphery to the center of each liver lobule which allows RE cells at the periphery first opportunity to remove substances from the blood. The peripheral pattern is greatly altered after 100 r whole body X-irradiation; radioautographs show a random distribution of $CrP^{32}O_4$ in the liver lobuli with little indication of a circular peripheral pattern. Apparently, the particles penetrate deeper into the liver sinusoids and are picked up closer to the central vein.

An additional observation was noted, namely, the number of cells containing $CrP^{32}O_4$ is reduced after X-irradiation according to counts of exposed areas per unit volume in radioautographs of liver. There is apparently no "regeneration," as assumed in Experiment A, because the number of RE cells per unit area was not the same after irradiation but was reduced when compared to controls. Despite this, the removal rate after irradiation was the same as before. From this evidence, it would appear that after irradiation there is an increased uptake of $CrP^{32}O_4$ by a smaller number of cells resulting in an unchanged disappearance rate of $CrP^{32}O_4$ from the blood stream.

However, the 100 r X-irradiation was followed by 0.1 ml. thorotrast "blockade," the $CrP^{32}O_4$ disappearance rate was prolonged (Experiment B). In this case, one reason for the longer removal time may be the reduced number of cells after irradiation. (One should not rule out the possibility

that there may be an altered circulation through the liver sinusoids after X-irradiation.)

The correlation of immunological processes and RES activity has been attempted for many years and is not well understood. The effect of X-irradiation upon immunological processes has been studied by a large number of investigators.[2, 18, 27] Recently, a brief radiosensitive period of the antibody response was noticed by Dixon, *et al.*,[7] shortly after whole body X-irradiation in rabbits. These authors considered it unlikely that the lack of antibody response after irradiation was caused by an RES damage, basing this conclusion on the data of others who have suggested that the RES is a radioresistant tissue. Our results indicate the opposite.

The "immuno-genetic" factor discussed in recent cancer research literature would seem to have some relation to RES activity. Following whole body X-irradiation, heterologous tumors can be transplanted successfully into rats.[29, 30, 31, 33] This change in tumor susceptibility caused by X-rays may possibly be explained in part by a changed RES activity, but too little is known at the present time to make any definite correlations.

Jacobson[17] has shown that lead shielding of exteriorized spleens enhanced very markedly the survival of mice after lethal doses of X-irradiation. On the basis of extensive studies he postulated the presence of a humoral factor, or factors, in the spleen which is responsible for recovery from radiation injury. With much smaller doses of X-irradiation in our studies, we noticed that the spleen seemed particularly radiosensitive.

Small doses of X-irradiation have been considered to stimulate RES activity.[15, 19, 28] However, our experiments indicate that the RES function will be definitely altered even by 25 r whole body irradiation and show a reduced RES selectivity after irradiation. These results, we feel, should be interpreted as an indication of impaired RES function. It is suggested, therefore, that the RES should be considered as a highly radiosensitive tissue rather than a radioresistant one.

SUMMARY

Rats were exposed to 25, 50, and 100 r whole body X-irradiation and reticulo-endothelial functions were tested by using the radioactive chromium phosphate technique. No change in the removal rate of chromium phosphate occurred during the four-week period after irradiation.

Induced thorotrast "blockade" was more effective in 100 r X-irradiated animals than in controls as measured by the disappearance rate of chromium phosphate from the blood stream, indicating that the reticulo-endothelial capacity was diminished by X-irradiation.

Organ distribution of chromium phosphate was altered after as little as 25 r. The liver showed an increased uptake with a maximum around the seventh day. The spleen took up markedly less chromium phosphate than controls during the first three days; and in rats receiving 100 r, the uptake was at a reduced level after fourteen days. The altered organ distribution of chromium phosphate was interpreted as a sign of impaired selectivity.

Implications of the above and possible immunological and radiobiological correlations have been discussed.

ACKNOWLEDGMENT

The authors wish to acknowledge gratefully the technical assistance of Miss Ulla Fogelberg.

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CARDIOVASCULAR STUDY UNIT

October 15, 1953

REMARKS OF CURRENT INTEREST ON CONGENITAL HEART DISEASE. By Paul Wood, Director, Institute of Cardiology, London, England.

The 720 cases of congenital heart disease summarized here may be divided into two groups: (i) those without shunt, and (ii) those in which shunting did occur (a) with and (b) without pulmonary hypertension.

About 90 per cent of coarctations of the aorta were visible on the plain PA chest film. Surgical repair is strongly recommended for all but the smallest. In 10 per cent of the group the defect was associated with a patent ductus.

In catheterizing patients with Ebstein's disease (tricuspid valve displacement with regurgitation) there seems to be a forbidding incidence of ventricular fibrillation. Auricular catheterization is useful in demonstrating borders of the right auricle to differentiate this condition from pericardial effusion. Mild cases of pulmonic stenosis are only of academic interest; they do not interfere with activity. Jugular pressure tracings in severe cases show a typical giant *a*-wave because of the strong right atrial contraction. Infundibular stenosis lacks the thrill in the third left interspace typical of pulmonic stenosis; a gradual increase of pressure is noted as the catheter is withdrawn from the pulmonary artery into the right ventricle.

Differentiating Eisenmenger's complex from patent ductus with pulmonary hypertension is easiest by catheterization since all ducti can usually be entered with a few trials. Many cases of so-called primary pulmonary hypertension may well be due to delayed closure of the ductus and persistence of the higher pressures thus established; this was seen in one case. Auricular defects, on the other hand, were characterized by low pulmonary pressures, even when six times as much blood passed through the lesser circulation as through the systemic circuit.

N. A. H.

YALE MEDICAL SOCIETY

October 26, 1953

CELLULAR MECHANISMS OF POTASSIUM TRANSPORT. By Gilbert H. Mudge, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York.

Slices of rabbit kidney cortex "leached" in isotonic saline to remove endogenous substrates were incubated in a Warburg apparatus. Before leaching, the potassium and sodium concentrations in the tissue were approximately equal—65 mEq./l. each—and after three hours of leaching they had changed to 25 mEq./l. of potassium and 120 mEq./l. of sodium. Incubation at 25° C. for one hour in a solution containing 10 mEq./l. of potassium and 138 mEq./l. of sodium resulted in restoration of the initial values against the concentration gradient. Oxygen is required by this reaction and 2,4-dinitrophenol is inhibitory, which suggests that the transfer of potassium and sodium depends on energy-rich phosphate bonds supplied by oxidative phosphorylation.

A second series of experiments employing radioactive isotopes of sodium and potassium demonstrated the existence of a continuous ion-exchange between cells and medium after establishment of the concentration gradients in the incubated tissue. Roughly 30 per cent of the intracellular potassium exchanges at a higher rate than the remainder. Under anerobic conditions, only 55 per cent enters the exchange at all. The rate or extent of the potassium exchange does not affect cellular sodium concentration, nor is the latter influenced by anerobic conditions.

Saline-washed mitochondria from rabbit liver were incubated at 25° C. in a medium containing 25 mEq./1. of potassium; the K/N ratio was found to be constant, but mitochondrial potassium continously exchanged with that in the substrate. Increasing amounts of dinitrophenol added to the medium did not alter the K/N ratio but did increase the potassium exchange rate after an initial depression. Exchange was also affected by varying orthophosphate concentrations.

These studies show that potassium is not found in a homogeneous state within cells. The different exchange rates prove the existence of at least two phases of intracellular potassium; part of the exchangeable potassium is bound to the mitochondria.

BRUCE DRAPER

ZOOLOGY-BOTANY JOURNAL CLUB SEMINAR

October 28, 1953

GENETIC ASPECTS OF SEX AND INCOMPATIBILITY IN FUNGI. By Haig P. Papazian, Plant Science Department, Yale University.

To an anatomist, sex is a problem of the evolution of reproductive organs; philosophy places sex in a different category, sometimes even considering it a luxury since sex, among many organisms, is not essential for reproduction.

Darwin failed to appreciate the multiplicity of varieties possible by particulate inheritance through sex. With only 100 mutations, for example, the number of genotypes possible in a diploid individual would far exceed the total number of men who have lived.

Sex occurs at random among bacteria; in insects and plants both sexual and asexual reproduction can take place, since environmental conditions determine which type is to predominate at a given time. Intermediate conditions (relative sex), as in certain algae, exist, but their genetics remains unsolved. Dioecious reproduction in animals can perhaps be attributed to their greater motility; this would offset the decreased fertility conferred by bisexuality. The multiple-allele heterothallism of the angiosperms and basidiomycetes represents a striking contrast in this respect.

Since reproduction between two different individuals (outbreeding) is evolutionally more desirable than inbreeding, the chance of reproduction occurring when two spores or gametes are united is greatest (100 per cent) with the hermaphroditic self-sterile organism, e.g. *Lumbricus* and *Helix*. Following next in efficiency are the Basidiomycetes (fungi). Whether or not a pair of spores from Basidiomycetes will prove compatible is determined by a factor governed by more than one pair of genes. A single muta-

tion can produce many new factors; where many alternatives exist, new factors arise which might be thought to be mutations. However, they can be shown to be the result of crossing over. An increase in the percentage of crossing over produces a rise in the degree of inbreeding, especially where the crossover frequency is greater than one per cent. Since it is never this great, the amount of possible variability is not significantly decreased.

Much similarity exists between the incompatibility of fungi and that of higher plants. In flowering plants the mutants produce fertility genes—rather strange, evolution-wise—but in fungi the mutants refuse to receive nucleii. Any type with such incompatibility factors as those of the fungi arising in a normal population would be at a distinct disadvantage. To be fixed, such genes would have to be present in a large majority of the individuals.

PETER KLOPFER

ZOOLOGICAL JOURNAL CLUB

November 4, 1953

CHOLINESTERASE IN THE AMPHIBIAN NERVOUS SYSTEM. By S. C. Shen, Department of Embryology, Yale University.

The rôle of acetylcholine and cholinesterase in the nervous system is well known. Another specific enzyme, cholinacetylase, forms acetylcholine from acetate and choline. The system $ACh \underset{CHa}{\overset{CHe}{\rightleftharpoons}} acetate + choline$ may be involved in more than synaptic transmission. We are concerned with the amphibian brain which has a high concentration of these compounds. Since they are not homogeneously dispersed, we examined their distribution pattern and obtained a fairly accurate picture using micro-dissection with manometric direct assay and histochemical localization.

Gross analysis indicated the concentration of cholinesterase in *amblystoma* larvae to be highest in the hindbrain and lowest in the forebrain. Using more refined methods, the least concentration existed in the hemispheres; all the important integrating centers—tectum, prectectal ganglia, nuclear isthmi, basal optic ganglia, the ganglia of the 3d, 5th, 7th, and 10th nerves, optic chiasma, and optic tract—had greater concentration.

In the frog, the retina and the synaptic field of the tectum exhibited matching synapse and enzyme bands which indicated a relatively high enzyme concentration in all important neural fields where there were synaptic junctions. The *CHe* specific activity varied directly with the functional activity of the developing optic lobe. The enzyme was almost homogeneously distributed in the adult frog tectum, but not in the larva where the greatest concentration was anterior and the least, posterior. By histochemical methods we noted that nerve endings first appeared at the anterior tectum; we concluded that the presence of the enzyme was closely correlated with nerve development.

Extirpation of an eye or the transection of the optic nerve reduced the enzyme activity in the opposite lobe. Specific activity of *CHe* may be

reduced by 58 per cent but another enzyme, succinoxidase, which is not involved in synaptic transmission, underwent no change in activity. Eye extirpation of larvae reduced the specific enzyme activity in the affected lobe. The difference was smaller than in the adult and probably due to invasion of the lobe by other nerves; we felt this again indicated a functional correlation between enzymes and neurons.

PETER KLOPFER

November 11, 1953

RECENT PROGRESS IN STUDIES OF BIVALVE LARVAE. By Victor L. Loosanoff, U.S. Fish and Wildlife Service Biological Laboratory, Milford, Connecticut.

Biologists require special methods to study the ecological and physiological needs of larvae. A knowledge of Lamellibranch genetics, ability to distinguish individual species (and plankton from species), and study of larval pathology are vitally necessary.

Until four years ago, larvae died before reaching an advanced stage; once we succeeded in raising the organisms, we could identify larval species. The usual breeding period of northern Lamellibranchs is two or two and one-half months; since our work required constant access to spawn, we "ripened" eggs artificially. Autumnal spawn were the result of an inhibition of the usual summer spawning and were produced by placing clams in cold water; we induced winter breeding by slowly raising water temperatures to 21-22° C. We have been able to induce spawning in our clams every six months for the past three years, and given the proper ecological conditions, this should be possible in nature, too, if one could provide an inactive period for gametogenesis and glycogen storage to take place. Chemical or mechanical stimuli released the eggs, but each species varied greatly in the ease with which it was accomplished. Abortions or abnormal spawn resulted when temperatures were too high, eggs were roughly handled, or spawning induced at an improper stage. These abnormal spawn produced abnormal larvae which varied considerably in size; this points to genetic differences which could be of commercial importance.

There was a direct relationship between egg concentration and size and growth rate of larvae. As the concentration rose, the growth rate fell; when the food concentration, regardless of the larval concentration, exceeded a certain figure, the larvae died. The growth rate was also dependent on temperature; the rate increased with a temperature rise from 18° to 30° C. Clam larvae have a rather narrow range of food requirements in contrast to the oyster. The larvae were not mechanical feeders as was formerly supposed; they showed both chemical and mechanical selectivity in feeding.

Color is an invalid criterion for species identification since it varies with the food eaten and can change within 30 minutes. We were able to use vital stains successfully; this may prove a valuable method for behavioral or ecological studies. Fungi are rare in sea water but larvae are susceptible to fungal infections; epidemics may kill a population in slightly more than 24 hours. Other than this, little is known of larval diseases.

PETER KLOPFER

BOOK REVIEWS

EPIDEMICS IN COLONIAL AMERICA. By John Duffy. Baton Rouge, Louisiana State University Press, 1953. xi + 274 pp. $4.50.

Using newspapers, diaries, letters, and the records of the Society for the Propagation of the Gospel and of some of the colonial governments, Duffy has tried to fulfill a definite need for a history of the major infectious diseases of colonial America. Since the sources are scattered and primarily written by laymen, much of the material is sketchy, and, although Duffy has been more conservative than some who have written on this period, some of his diagnoses are open to question. He has concluded that, although smallpox and yellow fever produced the most consternation, malaria, dysentery, and respiratory diseases in the long run caused the greatest social and economic loss. (He has not considered tuberculosis.) In the absence of adequate statistical material, however, the relative mortality of the various diseases remains largely a matter of speculation on the basis of personal impressions.

Unfortunately, Duffy has made numerous errors of fact and, in the opinion of this reviewer, of interpretation. He has offered broad generalizations without adducing sufficient evidence to support them. Since he has dug out much useful information and many details from original sources, persons particularly interested in the field may, with care, use the book profitably. But Duffy has tried to do too much. Considerably more research is necessary before anyone can write a comprehensive and accurate history of infectious diseases in the American colonies.

JOHN B. BLAKE

DISORDERS OF THE CIRCULATORY SYSTEM. Edited by Robert L. Craig. A symposium presented at the twenty-fourth graduate fortnight of the New York Academy of Medicine, 1951. New York, The Macmillan Company, 1952. xx + 301 pp. $5.50.

This book is a collection of the papers presented at the third graduate fortnight in two decades on disorders of the circulatory system. It is the first to be published. It is unfortunate that the others were not similarly treated. The subjects range from intracellular lipid metabolism to circulatory responses to life situations. The fact that such a wide coverage is attempted is not as amazing as that it is covered so well.

In the blurb on the dust-jacket the publisher selects two from the nineteen contributions as the most important. I must admit that it would be difficult for me to make a similar selection without first very carefully defining my frame of reference. I believe that probably every paper in this volume is potentially important to the medical man—clinician, researcher, or student.

Each subject is covered by an eminent leader in his particular field of cardiovascular study, and the list of contributors reads like a Who's Who in cardiovascular research. The price of this 301-page collection is $5.50 and is not unreasonable, even in the unfortunate absence of an index.

J. M. Q.

STEDMAN'S MEDICAL DICTIONARY. Edited by N. B. Taylor and A. E. Taylor. Baltimore, Williams and Wilkins Co., 1953. 18th rev. ed. xliv + 1,561 pp. $11.50.

The eighteenth edition of this standard, comprehensive dictionary contains several thousand new words and over 500 new illustrations. Like the previous editions, the eighteenth is an excellent medical dictionary.

The vocabulary includes "words used in medicine . . . dental, veterinary, chemical, botanical, electrical, life insurance and other special terms." The editors have excluded trade names. However, many personal names are included.

The treatment of each word includes spelling, syllabication and hyphenization. The system of pronunciation is easy to understand, and there are alternative pronunciations as in the case of "jĭ (gi, gī)ne-koló-je." Old disease names are given as well as some obsolete usages. The definitions are clear and adequate, and the illustrations further elucidate the definitions. Many abbreviations are included.

Among the special features are a 36-page section on medical etymology, 22 plates and 54 tables in the main text, and a useful Appendix containing equivalent tables, symbols, abbreviations, and other useful information.

FREDERICK G. KILGOUR

RESPIRATION IN PLANTS. By Walter Stiles and William Leach. Third Edition. New York, John Wiley and Sons, Inc.; London, Methuen and Co. Ltd., 1952. 172 pp., 10 diagrams. $2.25.

The new edition of this excellent monograph has much the same plan as the second. There are four chapters—a short introductory chapter, containing an historical account of the subject, definitions of terms, and methods, followed by three longer chapters, on aerobic respiration, anaerobic respiration, and respiratory mechanisms. While there are no extensive changes in the first three chapters, there are significant additions, such as the paragraphs on manometric methods in the first, and the enlargement of the part of the third chapter which concerns the effects of external factors on the intensity of respiration.

The last chapter has been entirely rewritten. It is organized into six sections: the connection between fermentation, anaerobic respiration, and aerobic respiration; the respiratory substrate; the course of fermentation and anaerobic respiration; the course of aerobic respiration (this includes a description of the oxidizing enzymes found in plants); the Pasteur effect and oxidative anabolism; and last, energy transfer in respiration. This list is sufficient to indicate that the chapter is not simply an exposition of certain biochemical pathways. This biochemical knowledge is not treated as the end in itself, but is fitted into the pattern of the respiratory behavior of organisms. One does not feel that there is any oversimplification, yet there is not the confusion of a mass of details.

To the student, perhaps the most important thing about this book is that it so clearly demonstrates the logical development of the major concepts and facts by experiment, hypothesis, and experiment. Not all aspects of respiration are included, but the basis is established for understanding the others.

The manner of expression in this book is far superior to that found in most texts or scientific papers. It is not only economical and unambiguous, but has a certain elegance. The reviewer feels that from the point of view of content and style, one can obtain from this book a maximum amount of information for a given amount of concentration.

R. R. L. G.

CANCER AS I SEE IT. By Henry W. Abelmann. New York, Philosophical Library, 1951. 100 pp. $2.75.

Cancer includes carcinoma, sarcoma, pernicious anemia, Paget's disease, rodent ulcer, "cancer granuloma," Hodgkin's disease, and mycosis fungoides and is a different expression of a single infectious disease; these forms arise from tissue cells whose walls have been weakened by certain agents to permit invasion of the cancer mold fungus, the pleomorphic germ-virus. This is the author's definition and statement of the etiology of the disease. No significant evidence is offered to support these theses and no bibliography included. Analogy is frequently substituted for fact and facts are incorrect in many instances. There appears to be no order or plan of presentation; the material is fragmentary, scrambled, and often contradictory. The author's purpose was to give a simple account of a large amount of material with the hope of encouraging research on the infectious nature of cancer.

J. K. R.

GENERAL GENETICS. By Adriam M. Srb and Ray D. Owen. San Francisco, W. H. Freeman and Company, 1952. 651 pp., 182 illus., 3 color plates, 62 tables. $5.50.

This book is a publication in the series of biological texts edited by G. W. Beadle, R. Emerson, and D. M. Whitaker. The text introduces at first the concept of the gene from which the basic ideas of Mendelism and the chromosome theory of inheritance are developed. Sections on biochemical and developmental physiological genetics follow and the nature of the gene is then discussed again. The last part of the book is devoted to problems concerning genetics of populations, questions of evolution, and of applied genetics. The examples chosen are taken from the genetics of both plants and animals.

The book is clearly written and well organized, with schematic drawings and photographic illustrations which are an integral part of the text. Each chapter is followed by a summary, a list of references and source material, and questions arranged in order of increasing complexity which are helpful both to student and teacher.

The decision of the author not to present an historical development of the subject matter could have been compensated for by a more comprehensive list of sources relevant to the historical background. This might well have included other than American and English sources. Despite this, the text can be highly recommended.

KRAFT VON MALTZAHN

BIOCHEMISTRY FOR MEDICAL STUDENTS. 5th ed. By William Veale Thorpe. Philadelphia, J. B. Lippincott Co., 1952. 503 pp. $6.00.

The title of the book reveals its purpose and nature. It is written for medical students who shun the more complete and heavier tomes of biochemistry and disdain outlines of the subject that read like telephone directories. In short, it is a clear and well-written introduction to biochemistry. Worthy attempts are made to correlate material, to provide clinical examples, and to make the subject as palatable and interesting as possible to medical students, yet conserving a modicum of experimental work.

This fifth edition differs substantially from the fourth. The chapters on metabolism have been largely rewritten "in order to place the tricarboxylic acid cycle in proper perspective as the final metabolic pathway common to amino acids, carbohydrates and fats." Other chapters also have been revised and rewritten.

The book is well organized. A preliminary review of some physicochemical principles, encompassing a good discussion of the colloidal state, precedes classification of carbohydrates, fats, and proteins and the chapters on enzymes and blood. Part two, concerning metabolism, the rôle of vitamins and hormones in metabolism, etc., is preceded by a discussion of digestion and absorption and a chapter on the use of isotopes in biochemical investigation. Part three pertains to principles of nutrition and a more than adequate treatment on the nature and composition of common foods.

No references are given, which is unfortunate when controversial statements are proffered without amplification, such as "the treatment of cases of human muscular dystrophy with the vitamin [E] has met with some success particularly in children, although the recovery is very much slower than with animals" (page 381); or, "the beneficial effects of glutamic acid given in mental diseases have been attributed to activation of choline acetylase" (page 192).

A useful bibliography of textbooks, monographs, and special articles of interest is to be found in the appendix.

ION GRESSER

THE ALGAE OF ILLINOIS. By Lewis Hanford Tiffany and Max Edwin Britton. Chicago, The University of Chicago Press, 1952. 407 pp., 108 plates. $10.00.

In 1944 Max E. Britton's *A catalog of Illinois Algae* was published as number two of the "Northwestern University Studies in the Biological Sciences and Medicine." *The Algae of Illinois* is the second in a series

intended to "include descriptions, keys, illustrations, and habitat analysis for the algal groups of Illinois." This volume carries the work up to, but does not include, the habitat analysis.

A six-page introduction briefly describes the Illinois terrain, characterizes the seven algal divisions, and gives a brief key to the classes, which are described again in detail in the text. The arrangement of the table of contents is particularly useful, because it is carried down as far as the families and keys to genera, which often saves one time thumbing the index. Every second or third page of the book is a plate, so that the illustrations are seldom far from the descriptions. As is usual in this sort of book, the figures are only detailed enough to show the necessary distinguishing features of the organisms. They are excellent in the opinion of this reviewer. The keys and descriptions of the taxonomic groups are clear and follow recent usage. There is an adequate glossary.

It is fair to ask how well such a book represents the algae as a whole, quite apart from how comprehensively it describes the algal flora of Illinois. Of the roughly 500 genera of fresh water algae now known to be in the United States, 178 genera, including 962 species and forms, are listed in the "Catalog," and a few more were added before this volume was published. This is representative enough to make the book useful in any region having habitats like those of Illinois. For identification of an organism as far as the species, one would often have to refer to monographs of the genera. For example, over 320 forms of *Chlamydomonas* have been described, but only one has been positively identified in the Illinois flora. The monographic references to the genera are given in the "Catalog," not in this volume, but the bibliography contains enough general references so that the monographs can be found even if the "Catalog" is not available. However useful this book may be as a guide to identification, its greatest value lies in the fact that it is part of a systematic effort to know the algal flora of a region. This kind of painstaking work is necessary for progress in ecology.

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*Kuhn. H.S.: Tr. Am. Acad. Ophth., p. 432. (March-April) 1951.

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